



**QUEEN'S  
UNIVERSITY  
BELFAST**

## **Microbicide Vaginal Rings: Technological Challenges and Clinical Development**

Malcolm, R. K., Boyd, P. J., McCoy, C. F., & Murphy, D. J. (2016). Microbicide Vaginal Rings: Technological Challenges and Clinical Development. *Advanced Drug Delivery Reviews*, 103, 33-56.  
<https://doi.org/10.1016/j.addr.2016.01.015>

**Published in:**  
Advanced Drug Delivery Reviews

**Document Version:**  
Peer reviewed version

**Queen's University Belfast - Research Portal:**  
[Link to publication record in Queen's University Belfast Research Portal](#)

**Publisher rights**  
© 2016 Elsevier. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <http://creativecommons.org/licenses/by-nc-nd/4.0/> which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

**General rights**  
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**  
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact [openaccess@qub.ac.uk](mailto:openaccess@qub.ac.uk).

# **Microbicide Vaginal Rings: Technological Challenges and Clinical Development**

R. Karl Malcolm\*, Peter J. Boyd, Clare F. McCoy, Diarmaid J. Murphy

School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK.

\*Corresponding author / reprints: R. Karl Malcolm, Tel.: +44 (0)28 9097 2319;

Fax: +44 (0)28 9024 7794, E-mail: k.malcolm@qub.ac.uk

**Running Title:** Microbicide Vaginal Rings

**Keywords:** vaginal rings; HIV microbicides; antiretrovirals; HIV prevention; controlled release; adherence; vaginal drug delivery

## **ABSTRACT**

Vaginal rings (VRs) are flexible, torus-shaped, polymeric devices designed to sustain delivery of pharmaceutical drugs to the vagina for clinical benefit. Following first report in a 1970 patent application, several steroid-releasing VR products have since been marketed for use in hormone replacement therapy and contraception. Since 2002, there has been growing interest in the use of VR technology for delivery of drugs that can reduce the risk of sexual acquisition of human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS). Although no vaginally-administered product has yet been approved for HIV reduction/prevention, extensive research efforts are continuing and a number of VR devices offering sustained release of so-called 'HIV microbicide' compounds are currently being evaluated in late-stage clinical studies. This review article provides an overview of the published scientific literature within this important field of research, focusing primarily on articles published within peer-reviewed journal publications. Many important aspects of microbicide-releasing VR technology are discussed, with a particular emphasis on the technological, manufacturing and clinical challenges that have emerged in recent years.

## 29    **CONTENTS**

### 30    1. Introduction

### 31    2. Microbicide-releasing vaginal rings

#### 32        2.1. Historical overview of vaginal ring technology

#### 33        2.2. Types of vaginal rings for microbicide delivery

#### 34        2.3. Material selection

##### 35            2.3.1. Silicone elastomers

##### 36            2.3.2. EVAs

##### 37            2.3.3. Polyurethanes

##### 38            2.3.4. Other materials

#### 39        2.4. Mechanisms of microbicide release from vaginal rings

##### 40            2.4.1. Drug release from matrix-type rings

##### 41            2.4.2. Drug release from reservoir-type rings

##### 42            2.4.3. Drug release from pod insert-type rings

##### 43            2.4.4. Combination swelling and permeation controlled release systems

##### 44            2.4.5. Osmotically controlled release systems

#### 45        2.5. Manufacturing approaches

##### 46            2.5.1. Thermosetting rings

##### 47            2.5.2. Thermoplastic rings

#### 48        2.6. Microbicide candidates

##### 49            2.6.1. Non-specific microbicides

##### 50            2.6.2. Antiretroviral microbicides

#### 51        2.7. Combination microbicide and multipurpose prevention technology (MPT) rings

52	3. Challenges moving forward
53	3.1. Formulation and delivery of biomacromolecular microbicides
54	3.2. Manufacturing issues and scale up
55	3.3. Cost
56	3.4. Acceptability and Adherence
57	3.5. Correlating <i>in vitro</i> release with <i>in vivo</i> pharmacokinetics
58	4. Conclusions

59    **ABBREVIATIONS**

- 60    ACV – acyclovir
- 61    AIDS – acquired immunodeficiency syndrome
- 62    API – active pharmaceutical ingredient
- 63    ARV – antiretroviral
- 64    AZT – zidovudine
- 65    Boc-LBA – Boc-lysinated betulonic acid
- 66    CG – carrageenan
- 67    DMPA – depot medroxyprogesterone acetate
- 68    DPV – dapivirine
- 69    DRV – darunavir
- 70    E2 – estradiol
- 71    EE – ethinylestradiol
- 72    ETN – etonogestrel
- 73    EVA – ethylene vinyl acetate copolymer
- 74    FDA – U.S. Food and Drug Administration
- 75    GMP – good manufacturing practice
- 76    GRFT – griffithsin
- 77    HAART – highly active antiretroviral therapy
- 78    HIV – human immunodeficiency virus
- 79    HRT – hormone replacement therapy
- 80    HSV – herpes simplex virus
- 81    HPEU – hydrophilic polyether urethane
- 82    HPMC – hydroxypropylmethylcellulose
- 83    HSV – herpes simplex virus
- 84    IgG – immunoglobulin G
- 85    IPM – International Partnership For Microbicides

86	IPA – isopropyl alcohol
87	IVIVC – <i>in vitro-in vivo</i> correlations
88	LNG – levonorgestrel
89	mAb – monoclonal antibody
90	MIV-150 – Medivir-150
91	MIV-160 – Medivir-160
92	MPT – multipurpose prevention technology
93	MTN – Microbicide Trials Network
94	MVC – maraviroc
95	N9 – nonoxynol-9
96	NES – nestorone
97	NRTI – nucleoside reverse transcriptase inhibitor
98	NNRTI – non-nucleoside reverse transcriptase inhibitor
99	NVP – nevirapine
100	PCL – polycaprolactone
101	PD – pharmacodynamic
102	PDMS – polydimethylsiloxane
103	PEU – polyether urethane
104	PK – pharmacokinetic
105	PI – protease inhibitor
106	Pt – platinum
107	RTV – room-temperature vulcanising
108	SE – silicone elastomer
109	SHIV – simian human immunodeficiency virus
110	SQV – saquinavir
111	STI – sexually transmitted infection
112	SVF – simulated vaginal fluid

- 113 TDF – tenofovir disoproxil fumarate
- 114 TFV – tenofovir
- 115 TPU – thermoplastic polyurethane
- 116 USP – United States Pharmacopoeia
- 117 VR – vaginal ring
- 118 ZA – zinc acetate



## 1. Introduction

In 1983, following two years of increasing number of reported cases in the United States (U.S) of severe immune deficiency among gay men and infants receiving blood transfusions, scientists first identified the human immunodeficiency virus (HIV) as the retrovirus that causes acquired immune deficiency syndrome (AIDS). By 1987, three biomedical strategies were at the forefront of developments to treat or prevent HIV infection. In March 1987, the U.S. Food and Drug Administration (FDA) approved the first antiretroviral (ARV) drug, zidovudine (AZT), for treatment of HIV by reducing replication of the virus. In August 1987, the FDA sanctioned the first human testing of a candidate vaccine against HIV. Later the same year, the FDA declared HIV prevention as a new indication for male condoms.

Fast-forward three decades and, despite the tremendous advancements in our scientific knowledge and understanding, the HIV/AIDS pandemic remains one of the most serious global public health crises of our time. The latest (2014) global statistics for HIV/AIDS estimate 37 million people living with HIV, 2 million new infections annually, and 1.2 million deaths in 2014 from AIDS-related illnesses [1]. Sub-Saharan Africa remains the hardest hit region, accounting for more than 70% of people presently living with HIV/AIDS.

Development of a safe and effective HIV vaccine has proven very difficult. Ideally, an effective HIV vaccine should induce powerful and durable immunity capable of preventing infection in healthy individuals and/or reducing viral replication and viral load in infected individuals with the aim of slowing or halting disease transmission and progression. To date, more than 250 clinical trials of HIV vaccine candidates have been completed or are presently being conducted;

only six of these candidates have reached late-stage clinical testing, and none have demonstrated significant efficacy [2].

With consistent and correct use, male latex condoms can reduce the risk of heterosexual transmission of HIV by more than 70% [3–5]. However, despite widespread and often aggressive promotion, condom use has not reached a sufficiently high level to impact rates of HIV acquisition in Sub-Saharan Africa. One reason lies with gender-power imbalances, resulting in women not always being able to negotiate condom use with male partners. For example, African men are more likely to refuse condom use when there are large differences in age between them and their female partners, if they are married, when they have multiple sexual partners, and where there is no communication about HIV/AIDS between them and their partners [6]. Female condoms, widely promoted as a female-controlled alternative to male condoms, have failed to gain acceptance, despite the introduction of new types [7–10].

On a more positive note, increased access to highly active antiretroviral therapy (HAART) means that an AIDS diagnosis is no longer a death sentence for millions of people. Today, 28 FDA-approved ARV drugs are available for treatment of HIV-1 infections [11]. These drugs are mainly classified into six distinct types based on their mechanism of action: nucleoside-analog reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors, protease inhibitors (PIs), fusion inhibitors and co-receptor antagonists. As of March 2015, 15 million people living with HIV, including 11 million in Sub-Saharan Africa, were accessing life-saving HAART, up from 13.6 million in June 2014 and only 300,000 in 2002, exceeding the targets set as part of the Millennium Development Goals [1]. Meanwhile, the number of people newly infected with HIV has fallen by 35% since 2000 and

global deaths due to AIDS have declined 42% since the peak in 2004. With this halting and reversing the spread of HIV/AIDS, and with continued effort and investment, the world is seemingly on track to end the AIDS pandemic by 2030 [1].

It is widely accepted that ARV treatment alone will not be able to curtail the HIV/AIDS pandemic. In the continued absence of an effective HIV vaccine, there is greater optimism about the clinical potential of HIV microbicides. HIV microbicides are pharmaceutical formulations administered vaginally (or rectally) to reduce sexual transmission of the virus. The concept of an HIV microbicide was first described in a 1990 commentary piece entitled ‘HIV Prevention: The Need for Methods Women Can Use’ [12]. Recognizing the limitations of behavior-modification strategies and use of condoms in reducing HIV infection rates, Stein strongly advocated research into new methods that women could use to prevent vaginal transmission of HIV. Of course, these ‘topical virucides’, as they were then called, would have to be acceptable to women in terms of convenience of use, safety and cost, as well as highly effective against the virus. A number of surfactant-type vaginal microbicides were tested in women during the 1990s (Figure 1), including a compound called nonoxynol-9 (N9). Most of these studies not only failed to protect women against HIV infection, but some actually increased HIV infection rates compared with a placebo product. Surfactant-type microbicides were subsequently abandoned. Next, the focus switched to various polymer molecules (Figure 1), whose negatively charged functional groups were shown in laboratory experiments to prevent the virus attaching to the immune cells. However, as with the surfactants, these polymer-based microbicides failed to provide protection in clinical studies, and once again, some increased the risk of infection.

The past five years has seen the microbicide field focus almost exclusively on more conventional small molecule ARV drugs, the same or similar drugs to those used since the 1980s for treating people already infected with HIV. A breakthrough came in 2010 when the first results emerged from the CAPRISA 004 trial [13]. For the first time, a vaginally-administered ARV gel product was shown to provide significant protection against HIV infection. A summary timeline describing key moments, and particularly major clinical activities, in the development of HIV microbicides is presented in Figure 1.

## **2. Microbicide-releasing vaginal rings**

The application of VR technology, first described and widely reported during the 1970s for vaginal delivery of contraceptive hormones (see Section 2.1), to the formulation of HIV microbicides was first proposed publicly at the 2002 Microbicides Conference in Antwerp, Belgium [14]. At that time, the microbicide field was almost exclusively focused on aqueous vaginal gel formulations containing non-specific microbicide candidates, such as the surfactant N9 and various polymer molecules (see Section 2.6.1), reflecting a lack of engagement and interest in the challenges of HIV prevention by basic scientists with expertise in drug design and formulation development. Today, semi-solid drug products, including gels, are the most common formulation type for vaginal drug administration. For example, of the 24 vaginal products currently marketed in the UK, 16 are either gels or creams, and only two are VR devices (Estring<sup>®</sup> and Nuvaring<sup>®</sup>). Since the beginning of the new millennium, a diverse range of formulation strategies for HIV microbicides has been investigated, including films, tablets, diaphragms, capsules, freeze-dried tablets, and nanoparticles [15]. However, ongoing concerns over poor user adherence associated with coitally-dependent gel products and a strong preference for formulations that offer continuous delivery of microbicide(s) over extended time periods has

resulted in a very significant shift in focus towards VR-based products that offer sustained/controlled release [15–17].

The first journal article describing a microbicide-releasing VR for HIV prevention was published in 2003 [18]. The matrix-type silicone elastomer ring contained the non-ionic surfactant N9, at that time a lead HIV microbicide candidate although soon to be overshadowed by evidence that in gel format it damaged the vaginal epithelium resulting in increased risk of HIV acquisition [19]. Two years later, the continuous, zero-order release of TMC120 (later renamed dapivirine, DPV) over 71 days from a core-type (also commonly referred to as ‘reservoir-type’) silicone elastomer VR [20] was described. This reservoir-type VR design was very similar to the marketed VR products Estring<sup>®</sup> and Femring<sup>®</sup>.

## **2.1. Historical overview of vaginal ring technology**

VRs are flexible, torus-shaped polymeric devices designed to provide sustained, long-term delivery of pharmaceutical agents to the vagina for clinical benefit. The first VR device for drug delivery was reported in a US patent application filed on 4<sup>th</sup> January 1968 and subsequently awarded on 8<sup>th</sup> December 1970 to the UpJohn Company [21]. These polymeric drug delivery ring devices, fabricated using silicone (polydimethylsiloxane, PDMS) elastomers, focused primarily on contraception and hormone replacement therapies (HRT). Since their inception in the 1960s, numerous ring designs containing various drug combinations have been proposed, tested and documented in the literature [21–26].

The first clinical trials of a VR were performed in the 1970s for the contraceptive progestin medroxyprogesterone acetate [23]. However, this early ring design, which consisted of a metal

spring over-molded by a silicone sheath (Figure 2A) caused numerous problems including erosion and ulceration of the vaginal epithelium [22]. Although first generation VR products were clinically effective, the devices were extremely rigid and inflexible making them prone to expulsion during normal daily activities. An early study of a levonorgestrel (LNG)-releasing ring device reported that 48 out of 139 female participants developed surface lesions/ulcerations, chronic inflammation and thinning of the vaginal epithelium [27]. These adverse effects were most likely the result of a combination of factors including (i) the geometry of the ring, (ii) the inflexible nature of the device causing localised pressure on the vaginal epithelium and (iii) epithelial thinning effects caused by the hormonal contraceptive agent. As a result thinner, more flexible, non-irritating VR designs with optimised geometries (outer diameter ranging from 50 to 58 mm; cross-sectional diameter ranging from 4 to 9.5 mm) were developed [28–32].

Despite initial enthusiasm for these new controlled-release VR devices, formulation issues and concerns regarding the safety of long-acting steroid releasing rings saw many ring development studies discontinued. To date, five VR products have reached marked – Estrin® (Pfizer Inc., USA) and Femring® (Actavis, UK) for hormone replacement therapy, NuvaRing® (Merck & Co, USA), Progering® (Laboratorios Silesia, Chile) for contraception, and Fertiring® (Laboratorios Silesia, Chile) for both pregnancy maintenance during *in vitro* fertilization and hormone replacement therapy in menopausal women. Clinical studies have shown a high degree of acceptability for VR devices over conventional semi-solid vaginal gels and creams [16,33–36].

Historically, silicone elastomers were the polymer of choice for fabrication of VR devices [20–23,37,38] owing to their lightweight, flexible nature and excellent biocompatibility. However, as VR designs have become increasingly more sophisticated a range of polymeric materials

including poly(ethylene-co-vinyl acetate) (EVA) [39–41] and more recently thermoplastic polyurethanes (TPU) have been used for the manufacture of these ring devices [17,26,42–44]. Materials for ring fabrication are discussed in more detail in Section 2.3.

## **2.2. Types of vaginal rings for microbicide delivery**

The application of VR technology to the delivery of HIV microbicide molecules having a broad range of physicochemical properties has led to very considerable innovation in new ring designs (Figure 2). Before 2006, VR designs being considered for microbicide delivery were mostly based on conventional matrix and reservoir-type systems, reflecting the design types used in marketed ring devices. However, the highly hydrophobic properties of EVA and silicone elastomer that had thus far proved successful for the formulation of hydrophobic, steroid drugs for estrogen replacement therapy and contraception were often not able to offer suitable permeation characteristics for many lead microbicide candidates. Also, microbicides in general, and ARVs in particular, do not generally possess the same clinical potency as steroid hormones, such that much larger doses (in the form of increased daily release rates) are required for efficacy. For example, while DPV has very similar physicochemical characteristics (e.g. molecular weight, partition coefficient, water solubility, etc.) to many steroid molecules, TFV and TDF are significantly more hydrophilic and therefore less capable of achieving clinically significant release rates from conventional VR designs that rely on permeation from hydrophobic elastomers as the primary mechanism of release. As a result, a raft of new ring designs – still largely based around silicone elastomer, EVA and TPU materials (see Section 2.3) – has emerged aimed at overcoming this permeation barrier, including segmented matrix, multi-core, segmented reservoir, rod/tablet insert, core-matrix and pod insert rings (Figure 2). A major impetus for continued innovation in ring design has been the growing interest in combination microbicide

[45] and multipurpose prevention technology products [46–49] (see Section 2.8), for which release characteristics need to be individually tailored for each drug molecule. The release mechanisms that govern these new designs are discussed in Section 2.4.

### **2.3. Material selection**

Drug-releasing VRs, comprising a combination of drug and device, are formally classified as combination products as defined in FDA documentation 21 CFR 3.2(e). Most commonly in VRs, the drug component takes the form of a potent ARV molecule and the device component comprises a polymeric ring device. Because combination products involve components that would normally be regulated under different types of regulatory authorities, and frequently by different FDA Centers, they raise challenging regulatory, policy, and review management challenges. Differences in regulatory pathways for each component can impact the regulatory processes for all aspects of product development and management, including preclinical testing, clinical investigation, marketing applications, manufacturing and quality control, adverse event reporting, promotion and advertising, and post-approval modifications [50].

Biological evaluation of polymeric medical devices that come into direct or indirect contact with the human body are covered by ISO-10993, “Biological Evaluation of Medical Devices Part 1: Evaluation 132 and Testing” [51]. The general principles of this ISO-10993 guidance also apply to combination products, such as drug-releasing VRs, although additional or modified testing may be required. According to the standard, VRs are classified as devices that contact the mucosal tissue, and for which a series of initial tests are mandated to evaluate biological effect. These can include tests of cytotoxicity, sensitization, vaginal irritation, systemic toxicity, subchronic toxicity, genotoxicity, and implantation, depending on the duration of mucosal



contact. A supplementary evaluation test for chronic toxicity is also required for drug-releasing VRs that are used for greater than 30-day duration.

To date, all marketed VRs and most prototype microbicide-releasing VRs are fabricated either by high-temperature reaction injection molding of medical grade silicone elastomers (Estring<sup>®</sup>, Femring<sup>®</sup>, Progering<sup>®</sup>, Fertiring<sup>®</sup>) or high-temperature extrusion of EVA (Nuvaring<sup>®</sup>). For example, The International Partnership for Microbicide's (IPM) DPV VR 004 is manufactured from Nusil's MED-4870 silicone elastomer. Certain grades of these polymeric materials meet the standards of United States Pharmacopeia (USP) Class VI materials (the most stringent of the six classes of plastic designation). In order to be compliant with USP Class V.e approval, test materials must pass the 'Systemic Injection Test' and the 'Intracutaneous Test'. Here, extracts of the test material in saline, alcohol in saline, polyethylene glycol (PEG 400), and vegetable oil, are injected into mice and rabbits and the animals' response to the sample extracts compared with a blank test. USP Class VI materials must pass both the USP Class V.e test plus an implantation test in which strips of the test material and a negative control are implanted in rabbits for a period of not less than 120 hr. Hemorrhage, necrosis, discolorations, and infections are macroscopically observed and degree of encapsulation is scored and compared with a negative control to determine test passage. Although USP Class VI testing is widely used and accepted in the medical products industry, some view it as the minimum requirement a raw material must meet to be considered for use in healthcare applications. USP Class VI testing does not fully meet any category of ISO 10993-1 testing guidelines currently used by the US FDA (General Program/Bluebook Memorandum G95-1) for medical device approval.

### *2.3.1 Silicone elastomers*

Silicone elastomers for use in medical and pharmaceutical applications are prepared through the chemical crosslinking of functionalised, linear, polydimethylsiloxane molecules. The most important chemical crosslinking mechanisms involve condensation-cure and addition-cure chemistries. Condensation-cure systems involve reaction between hydroxy-terminated polydimethylsiloxanes and a tetraalkoxysilane, resulting in the formation of the cured elastomer and an alcohol by-product (Figure 3) [52–54]. Although the chemistry of this SE crosslinking reaction is generally compatible with a very wide range of chemical functional groups, the alcohol produced can be problematic when an incorporated drug(s) is highly soluble in the alcohol [55,56]. Crosslinking of addition-cure SE systems relies on the platinum-catalysed hydrosilylation reaction between hydride- and vinyl-functionalised polydimethylsiloxanes (Figure 4). Usefully, no by-product is formed with this reaction. However, the platinum (Pt) catalyst is particularly sensitive to poisoning by certain chemical functional groups, most notably organotin, organosulfur and certain amine containing compounds. Medical grade silicone elastomers used in the fabrication of VRs are supplied as either restricted (limited to external use or short term implant applications  $\leq 29$  days) or unrestricted grades (for any application, including long term implantation  $> 29$  days). In general, they are supplied as two-part systems that need to be intimately mixed to initiate cure. Each part includes the silicone base material – a complex mixture of silicone polymers and a reinforcing filler (Table 1) – in addition to platinum catalyst, hydride crosslinker and cure inhibitor components (Table 2).

### 2.3.2 EVAs

EVAs are copolymers of ethylene and vinyl acetate that have a long history of use in drug delivery applications (Figure 5A). The vinyl acetate content (typically ranging from approximately 10–40%) and the molecular weight characteristics of the EVA material play a

major role in determining the mechanical properties, the ease of processing, and the drug release rates of the finished drug delivery device. The first controlled release drug delivery systems to be commercialized – Alza's Ocusert® (an ophthalmic insert releasing pilocarpine at a constant rate for treatment of glaucoma) and Progestasert® (an intrauterine implant providing constant rate of progesterone delivery) – were fabricated from EVA. More recently, Implanon®/Nexplanon® (a long-acting subdermal contraceptive implant releasing etonogestrel), Virtasert® (a ganciclovir eye implant for treating cytomegalovirus infection) and Nuvaring® (a combination contraceptive VR) are all fabricated from EVA. Despite the success of Nuvaring®, there have been only a small number of reports describing use of EVA for microbicide-releasing VRs. The reasons for this are unclear, although supply of medical grade EVA materials is often more constrained than for silicone elastomers (due to limited number of vendors). However, EVA polymers may offer certain advantages over silicone elastomer materials, including lower cost, a wider range of physicochemical and drug permeation properties due to variation in the vinyl acetate ratio, and the potential to produce devices with very thin (less than 100 µm) rate controlling membranes using extrusion processes [57,58]. The experimental NNRTI UC781 showed similar *in vivo* release rates and kinetics in rabbits following vaginal administration of ring segments fabricated from EVA, polyurethane and silicone elastomer [59]. A combination EVA matrix-type ring providing simultaneous release of the microbicide candidate UC781 and the contraceptive progestin levonorgestrel (LNG) has also been reported [40]. The Population Council are developing an EVA core-matrix ring for simultaneous delivery of Medivir-150 (MIV-150), carrageenan (CG), zinc acetate (ZA) and levonorgestrel (LNG) [60].

### 2.3.3 Polyurethanes

Alternative biocompatible thermoplastic materials, most notably thermoplastic polyurethanes (TPUs) [42,59,61–64], are also being evaluated for fabrication of microbicide-releasing rings, in order to extend material choice beyond the relatively hydrophobic silicone elastomers and EVA polymers. TPUs are multi-phase block copolymers formed by a step-growth polymerization reaction between diisocyanates, a low molecular weight diol and a high molecular weight diol (Figure 5B). The low molecular weight diol and the diisocyanate combine to form hard segments which contribute to the toughness and physical performance properties; the high molecular weight diol and the diisocyanate combine to form soft segments (responsible for the flexibility and elastomeric character). The TPU polymerization reaction combines the soft segments and hard segments into a linear backbone, giving a copolymer with bi-phasic properties. The hard and soft phases separate as a result of the strong hydrogen bonding between urethane units and/or the hard segment crystallization. By controlling the ratio of hard to soft segments, TPUs offer a wide range of physicochemical and drug release properties useful in developing VR formulations optimized for different types of drug actives. For example, TPUs are available in both hydrophobic and hydrophilic grades, and both have been reported in the literature for VR fabrication. CONRAD's dual-segment, multipurpose prevention technology VR releasing TFV and LNG is fabricated from two different polyurethane materials (Lubrizol's Tecoflex™ and Tecophilic™ polymers; GMP versions are sold under the Pathway™ brand), one for each segment and selected to optimize permeation of the actives [61]. TPU VRs are generally fabricated by blending/compounding the drug with the polymer followed by hot-melt extrusion processes (although injection molding is also possible). Depending upon the properties of the drug in the polymer, the drug may associate mostly with the soft segments or with both the soft and hard segments.

#### 2.3.4 Other materials

Microbicide-release vaginal rings fabricated from hydrogel materials have been reported [65–67]. Han et al. described rings composed of biosoluble acacia gum or nonbiodegradable hydrogel of 2-hydroxyethyl methacrylate (HEMA) and sodium methacrylate (SMA) [P(HEMA- co-SMA)] for the *in vitro* release of AZT and various non-hormonal contraceptives [65]. In a follow-up paper, the same technology was extended to release of dapivirine (TMC120), PMPA and Boc-lysinated betulonic acid (Boc-LBA) [66]. For each drug, *in vitro* release was maintained for no less than 15 and 28 days from the acacia gum and 2-hydroxyethyl methacrylate and sodium methacrylate rings, respectively, at concentrations higher than the minimum effective dose for HIV inhibition. The same group of researchers have more recently reported a biocompatible VR composed of a nanoporous poly(diols citrate) elastomer hydrogel for the delivery of non-hormonal contraceptives and anti-HIV agents [67]. Following synthesis of the prepolymer (by mixing and heating a mixture of 1,8-octanediol and citric acid) and subsequent mixing/processing with the active agents, the viscous polymer mixture was poured into a ring mold and heated at 80 °C for 4 days to form the final ring device. Use of organic solvents and a complex and protracted manufacturing method are likely to restrict the scope and practicality of this VR technology.

Biodegradable polycaprolactones (PCLs) are commonly used for production of biomedical implants and drug delivery devices in the form of films, micro- and nanoparticles. Pertinent to this review, microporous PCL vaginal insets offering 30-day controlled release of TFV have recently been reported [68]. Unlike more conventional polymer materials used to construct ring devices, preparation of these prototype rings involves dissolving/dispersing the polymer/drug in acetone followed by a methanol extraction process, resulting in a relatively flexible porous insert. Such a solvent method is not safe, practical or scalable for commercial ring manufacture.

However, PCL is amenable to hot-melt injection molding and extrusion. The porous nature of these materials, which facilitates fluid uptake, offers an alternative mechanism of release for those microbicidal drugs that are not amenable to permeation control polymer systems.

## **2.4. Mechanisms of microbicide release from vaginal rings**

All of the VR products currently marketed (Estring<sup>®</sup>, Femring<sup>®</sup>, Nuvaring<sup>®</sup>, Fertiring<sup>®</sup> and Progering<sup>®</sup>) and the DPV Ring-004 in Phase III clinical testing rely on release of the active from the device via a permeation-controlled mechanism [20]. This permeation process can be considered as three discrete and consecutive steps – drug solvation in the surrounding polymer, molecular diffusion of the solvated drug molecules within the polymer, and partition of the drug from the surface of the ring into the surrounding release medium [25]. The driving force behind the permeation mechanism of drug release from VRs is passive diffusion down concentration gradients that exist from within the device to the fluid in the vaginal vault. The nature of the release profile observed *in vitro* depends upon the ring design and allows differentiation of ring type based on characteristic release profiles e.g. matrix and reservoir-type rings. Recently other mechanisms of drug release beyond permeation control have been investigated including combination swelling and permeation controlled systems, pod-insert type systems and osmotically controlled systems.

### **2.4.1 Drug release from matrix-type rings**

In the simplest matrix-type ring design, drug is homogeneously dispersed throughout the entire ring body (Figure 2B). For most drug compounds, solubility in the polymer is lower than the amount of drug present, such that drug is present in both the solvated and solid (usually crystalline) states. Upon placement of the ring into a release medium, solvated and solid drug

initially present at the outer surface of the ring will diffuse/dissolve into the surrounding fluid, giving rise to the so-called ‘burst effect’ [25,55]. Once this very outermost layer of drug has been released, other solvated drug molecules from within the bulk of the VR will diffuse to the ring surface and partition into the surrounding fluid. The solubility sites within the polymer that have been depleted due to drug release are then replenished by dissolving of further drug molecules from the solid drug particles dispersed within the matrix. As a result of these processes, a series of equilibria are established – solid drug in polymer  $\rightleftharpoons$  dissolved drug in polymer  $\rightleftharpoons$  drug in release medium / vaginal fluid – which are maintained so long as excess solid drug is present within the device. Given sufficient release time, an advancing drug-depletion zone forms with matrix-type VRs [69].

Usually, the rate-limiting step in release of drugs from matrix-type VRs is molecular diffusion of the drug through the polymer, which, under sink condition, is commonly modelled by the Higuchi equation [70,71]. Given that the Higuchi model was originally derived for planar matrices rather than a torus-shaped system [72], Helbling et al. present an alternative and more accurate mathematical model for controlled release of drugs from torus-shaped matrix-type devices [72]. Representative daily and cumulative release profiles for matrix-type VRs are presented in Figure 6. There is characteristic reduction in the daily release as the depletion zone boundary recedes into the ring thereby increasing the diffusional path of dissolved drug to the surface of the ring. The release rate is dependent upon the drug solubility in the polymer, the diffusion coefficient of the drug in the polymeric material of the ring, the drug loading and the ring surface area.

Through judicious choice of release medium, matrix-type rings can give rise to both diffusion-controlled and partition-controlled release mechanisms [73]. Diffusion-controlled release predominates when the drug solubility in the release medium is sufficiently high [74]. In this scenario, the shape of the release profile is relatively insensitive to the partition coefficient and solubility of the drug considered. However, as the solubility of the drug in the release medium falls the mechanism of release will shift to a partition-controlled one [74]. Here the cumulative drug release profile is linear with time, corresponding to zero-order release. This can be thought of as having a constant supply of dissolved drug in the polymer waiting to be released into the surrounding fluid with the rate-limiting step being the partitioning of the drug into vaginal fluid/release medium rather than diffusion through the polymer matrix. The drug release process is then a function of the partition coefficient of the drug between the polymer and the fluid surrounding the ring.

The 25 mg DPV Ring-004 – the most advanced microbicide ring, currently in Phase III clinical trials – has a matrix design [75,76]. Other examples include the EVA rings containing either MIV-150 [77] or MIV-160 developed by the Population Council [78], both of which exhibit partition-controlled release into an acetate buffer with surfactant system and the hot melt extruded polyether urethane ring containing UC781 [79]. Combination matrix-type rings containing more than one microbicide have also been investigated [80,81].

#### *2.4.2 Drug release from reservoir-type rings*

In simplest form, reservoir-type VR designs comprise a central drug-loaded polymer core (again, drug is generally present in both the dissolved and solid state) surrounded by a non-medicated polymeric rate-controlling membrane (Figure 2E–G). For example, each of the marketed VRs



Nuvaring<sup>®</sup>, Estring<sup>®</sup> and Femring<sup>®</sup> is a reservoir design, although the length of the drug-loaded core varies between the devices. Most commonly, release is governed by a permeation mechanism involving dissolution and diffusion of drug molecules in the polymer materials from which the ring is fabricated. Drug release rates from reservoir VRs are typically constant with time, consistent with zero-order kinetics. Sometimes, depending on the drug/polymer combination, the manufacturing conditions and the stability conditions, a lag or burst effect can be observed during the initial release period (Figure 6). As only dissolved drug can migrate through the ring structure from the core to the periphery, the rate of release is controlled by the fixed thickness of the membrane layer. Constant rate of drug release will continue until the solid drug within the core becomes depleted. A fixed diffusional path length gives rise to a fixed release rate dependent upon the rate of partitioning into the surrounding fluid and the size of the sheath layer. Drug release kinetics in this case are controlled by the thickness of the rate-controlling membrane and the relative partition coefficient of the drug between the polymer and the release medium [82]. Owing to their design, reservoir-type devices offer lower release rates compared with matrix-type rings. Examples of reservoir-type microbicide VRs include the 90-day TFV ring [83], the tenofovir disoproxil fumarate (TDF) reservoir ring [63,84] and the DPV reservoir rings tested by IPM [56,85].

#### *2.4.3 Drug release from pod insert type rings*

Pod insert VRs comprise compacted drug powder inserts coated with a semipermeable polymer and embedded in a polymeric (often silicone elastomer) VR body [86–89]. This design offers pseudo zero-order release profiles and can be used to deliver a broad range of compounds including hydrophilic and macromolecular actives. Drug release, which occurs by permeation through a delivery window in the ring body, can be readily altered by changing the window

diameter, altering the amount and composition of the core polymer coating, or by increasing or decreasing the number of pods per ring [86]. The pod ring has been investigated for the delivery of the hydrophilic drugs TFV and ACV [89,90], and simultaneous delivery of five different drugs – TFV, nevirapine (NVP), saquinavir (SQV) and the hormonal contraceptive combination etonogestrel (ETN) and ethinyl estradiol (EE) [91]. The release of antibodies has also been investigated (see Section 3.1) [92].

#### *2.4.4 Combination swelling and permeation controlled release systems*

TPUs have been used in the manufacture of various ring types, including matrix [42,44,79], reservoir [44,63] and segmented designs [43,61] (Figure 2). Segmented polyurethane rings, comprising a water swellable polyurethane segment and non-water swellable polyurethane segment within the same ring device, are useful for release of compounds with very different hydrophilicities. For example, a segmented ring has been reported for simultaneous delivery of TFV and DPV [43]. Depending upon microbicide solubility in the release media, the release mechanism can be diffusion controlled or partition controlled [42,79]. The use of a water swellable polymer requires that polymer swelling be taken into account for the release mechanism [44]. Variations on the segmented ring design have also been described, including the use of hollow tube like cores with osmotically active excipients present to encourage water ingress and drug release [83] and segmented dual reservoir-type designs (Figure 2G) [61]. A dual-segment version of this latter ring type has been developed as an MPT device offering release of TFV from one segment and LNG from the other [61].

#### *2.4.5 Osmotically controlled release systems*

A new core-matrix MPT ring containing four different APIs has recently been reported by the Population Council (Figure 2J) [60]. This VR comprises a compressed core containing the solid hydrophilic agents ZA (targeting HIV-1 and HSV- 2) and CG (targeting HPV and HSV-2). The core is embedded within a hot-melt extruded EVA ring body containing the hydrophobic antiretroviral MIV-150 and the hydrophobic progestin LNG. Pores drilled in the EVA ring allow fluid ingress to dissolve and release the ZA+CG from within the core. In this manner, different mechanisms control release of the various actives. The concept of using rings to hold inserts containing highly hydrophilic excipients to promote release of macromolecular drugs has also previously been reported [93]. Recently, a device utilizing polymer swelling for the controlled release of macromolecules has been reported [94].

## **2.5. Manufacturing approaches**

VR manufacturing techniques are dependent on the design, APIs, materials and production volume requirements of the device. For most microbicide-releasing VRs, the polymeric excipients used in their manufacture play a major role in controlling the release of drug(s), usually by limiting the rate of drug diffusion through the matrix body or a non-medicated layer. Thermosetting and thermoplastic polymers are the most commonly used materials used in the manufacture of microbicide-releasing VRs; thermosetting polymers cure irreversibly, while thermoplastic polymers can be thermally cycled. Summaries of the manufacturing approaches used for the different material categories are found in Figures 7 and 8.

### *2.5.1. Thermosetting materials*

Silicone elastomers are the main thermosetting polymer used in the manufacture of VRs. They are available in a variety of curing chemistries including condensation-cure (Figure 3), addition-

cure (Figure 4), room-temperature vulcanizing (RTV) and ultra-violet (UV). The chemistries of condensation-cure and addition cure systems are described in Section 2.3.1.

The basic manufacturing principles for condensation-cure silicone elastomer rings are the homogenous distribution of n-propylorthosilicate into a silicone elastomer base followed by addition of the active ingredient and thorough mixing (Figure 7). A tin catalyst is dispersed into the formulation and final forming operations are performed at temperatures typically above 100°C.

For drug molecules with non-reactive functional groups, addition-cure systems are the preferred option. Addition-cure silicone elastomers are two component systems (Part A and Part B) which are typically combined in a 1:1 ratio [20,38,56,69,80,95–97]. Active ingredients are usually dispersed in equal amounts into each elastomer component in separate batch mixing operations, and then these active mixtures are combined in appropriate ratios using an additional mixing operation before final forming, heating and curing of the ring.

At a laboratory scale, mixing of components and API into the elastomer base has been performed by hand, overhead paddle, planetary [80] and double-asymmetric centrifugal (DAC) mixing [80,96]. The selection of a suitable mixing method depends on the viscosity of the silicone component, the amount of excipients and/or API being added, the sensitivity of the components to processing conditions, batch size requirements and also the degree of scalability necessary for a given stage of product development. DAC mixing is capable of dispersing multiple APIs in silicone elastomer materials to allow the production of combination microbicide matrix-type VR formulations [80,81,96].

594

595 Given the relatively rapid kinetics of the crosslinking reaction for both condensation and addition  
596 cured silicone elastomer systems, final downstream mixing is ideally performed immediately  
597 before the product forming operation occurs. Working with small batches of premixed material  
598 can mitigate the problem of the elastomer curing prior to completion of final product forming but  
599 accepted best practice is the use of static mixing equipment to combine the incoming streams of  
600 components; just subsequent to forming. This contrast is highlighted again by Fetherston et al.  
601 [80] in their different approaches to producing R&D scale batches using a DAC mixer working  
602 with mixed batches of Part A and Part B API loaded elastomer compared to the method used  
603 during large scale manufacturing runs for stability trial samples where the two separately pumped  
604 A and B streams were combined using a static mixer, prior to being fed into an injection molding  
605 machine.

606

607 Silicone elastomer VRs are usually fabricated using injection molding processes. After final  
608 combination of all liquid components, material is transferred into an injection vessel that  
609 pressurizes and provides a mechanism of control over the ‘shot’, specifically the volume of  
610 material that flows into a mold tool containing a negative ring cavity. The mold tool is  
611 temperature controlled and heated to a set point that provides crosslinking of the silicone  
612 elastomer in the ring as rapidly as possible without causing detrimental effects to the initial  
613 injection – linked to ring quality, or degradation of API contained therein. The processing  
614 parameters that can be controlled during injection molding operations of liquid silicone  
615 elastomers and their potential effect on product quality have not been widely reported. Evidence  
616 from studies of Pt-catalysed silicone elastomer maxillofacial prostheses suggest that that low

temperature-long duration vs high temperature-short duration curing conditions produce no appreciable differences in material hardness but mold material could have an effect [98].

For silicone elastomer reservoir-type VRs, the drug-loaded core component is formed as for a matrix-type ring. This core is then placed into a mold that allows half of the core cross-sectional diameter to be covered in a non-medicated membrane of chemically-compatible silicone elastomer. When cured, the half-sheathed ring is removed and placed into a third mold assembly that allows the final part of the sheath layer to overmolded around the core, forming a full rate controlling membrane around the API loaded core [20]. There is increased complexity in the manufacture of silicone reservoir rings compared to their matrix counterparts due to the importance of centrally locating the core within the membrane to ensure consistent drug release rates are obtained. In addition a two or three step injection molding process is required that has implications for manufacturing costs.

Reservoir-type microbicide rings can also be manufactured by injection molding to contain partial-length cores (unpublished). Here, full cores are molded and the required segment size is cut from the full core, e.g. half, quarter etc. The overmolding process is then performed in the same manner as for full core, injection molded reservoir rings. A non-microbicide example of a silicone multi-core reservoir VR containing oxybutynin examined different fractional segment cores, using this approach to reduce the day one 'burst effect' observed in full-length cores [55].

An additional forming operation for silicone elastomer products is the extrusion of rod geometries. This approach is particularly useful when working with high drug loadings (> 40% w/w) in silicone elastomer systems, where viscosity of the silicone mixes exceed the capability of

the injection molding process. For silicone elastomer extrusion, drug-loaded material is conveyed using an Archimedean screw inside a temperature-controlled barrel. A circular die placed at the output from the extruder forms a rod of defined diameter and this is passed through a line of convection ovens or a static oven to cure the elastomer. One example of the use of API-loaded silicone rods is the Population Council's nesorone (NES) / EE ring [99] that has two separate rod inserted into a single ring device. Once active silicone rods have been extruded, they are cut to length and either overmolded with a compatible silicone or, as in the case of the NES/EE ring, inserted into a separately produced, non-medicated silicone ring body. Whilst this example is for a contraceptive ring application, the manufacturing techniques could equally apply for microbicide releasing VRs delivering two or more actives.

A subset of reservoir-type silicone VRs exists where the ring body acts as a non-medicated holder for active silicone cores. Examples include the Population Council's NES/EE VR, where the ring body is manufactured separately and the cores are added in a separate operation [99], and a tablet insert ring in which API-loaded capsules are inserted into an injection molded ring body manufactured using mold tooling that forms defined hole diameters traversing the cross section of the ring [93,100]. One advantage of this manufacturing approach is that thermally-sensitive actives, such as proteins or peptides, can be readily incorporated into ring devices without exposure to the elevated temperatures required to cure the silicone elastomers. A different type of pod ring has been developed using cropped, spherical pods of solid API coated with permeable and semi-permeable polymers. Early prototypes of the device were manufactured with a recess for pod insertion that was backfilled with silicone [86]. Delivery channels were mechanically punched through the base of the ring body. The pods were manufactured separately giving the capacity to deliver multiple API [91]. In recent advances to the manufacturing process, the

delivery channels were molded directly into the silicone sheath layer [101]. First, a ring body is injection molded with a recess for each pod that includes a cylindrical orifice or ‘delivery channel’ at the base, formed during the molding process - designed to control release rate of the individual API loaded pods. The pods are inserted into their recess and fresh silicone is used to backfill the recess, locking the pods in position and completing the ring profile.

### *2.5.2 Thermoplastic rings*

Thermoplastic rings differ from thermosetting VRs in their manufacturing approach, specifically in the steps required to create homogenous dispersions of API in the viscous thermoplastic melts. For matrix-type VRs, the API is first dispersed in the selected matrix polymer (powder or pellet form) before final forming operations occur (Figure 8). The types of equipment used to disperse API throughout the base polymer are Banbury type mixers and single/twin screw compounders suitable for melting the polymer and high shear/torque mixing. Another method used to disperse API into polymer prior to extrusion is solvent casting [42,43] with drug and polymer dissolved in suitable solvent then evaporated to form films that are subsequently chopped up and fed into the extruder. Extruders have the capability to provide a continuous output of API loaded rod, sized according to a mold or ‘die’ fitted to the output of the extrusion barrel. This rod can be used directly to create rings if it is cut, shaped into a torus and butt welded to form seam joint [42]. Also, with the advantage of simple thermo-mechanical jointing methods, it has been possible to combine segments of compatible API matrix rods to form segmented matrix rings containing multiple API [43]. Alternatively, as for DPV loaded EVA matrix rings, the rod can be cut into granules via pelletization and used in injection molding operations to form the final ring. Injection molded thermoplastic matrix rings have also been adopted for combinations of API for HIV and contraceptive function [40].



689

690 Co-extrusion is a widely-adopted method for manufacture thermoplastic reservoir VRs. Here,  
691 API is compounded in an extruder to provide a homogenous output of API+polymer, while an  
692 additional extruder provides a secondary stream of non-medicated polymer that is compatible  
693 with the polymer used in the active stream. The API loaded stream forms a rod that is coated with  
694 the non-medicated stream forming a core/sheath configuration with core diameter and sheath  
695 thickness dictated by the geometry of the die. The rod is cut into defined lengths, placed into a jig  
696 that bends it into a toroid and butt-welded to form a seam. A non-microbicide exemple of a  
697 commercial thermoplastic reservoir ring manufacture is Nuvaring [58,102]. Powdered EVA is  
698 mixed with the contraceptive hormones using a twin screw extruder and fed through a co-  
699 extrusion die, while a second extruder feeds non-medicated EVA to provide a rate controlling  
700 sheath layer thickness of approximately 100 microns.

701

702 Reservoir-type VR design principles have also been used to create microbicide loaded  
703 thermoplastic devices using polyurethane sheathes [83]. Hydrophilic polyurethane was extruded  
704 into a hollow tube configuration and the lumen filled with microbicide powder only or powder  
705 and glycerol/water combinations, end sealed using an induction weld. This straight piece of  
706 sealed tubing was then shaped in a ring die, annealed then the two ends of the ring were joined  
707 using a final induction weld. Different formulations of a combination microbicide/contraceptive  
708 hormone VR were fabricated from conventionally co-extruded LNG reservoir strands and hollow  
709 core design TFV strands [61]. To reduce leakage of LNG into the TFV reservoir strand, low  
710 permeability polymer caps were placed between the different reservoir segments, and all of the  
711 various joints were induction welded.

712

The Population Council has recently reported a multipurpose prevention ring containing API to prevent HIV, HSV, HPV in combination with a contraceptive hormone [60]. The device is a combination of matrix and core technologies in order to release both hydrophobic and hydrophilic actives. The outer body of the ring was formed in two halves using separate hot melt extrusion stages combining EVA, microbicide and contraceptive hormone. The first extrusion stage produced a semi-circular; half ring with a channel into which an inner core formulation using hydrophilic actives was formed. The second hot melt extrusion step produced the upper half of the matrix ring minus the channel and completed the ring profile. Orifices of defined diameter were drilled through the matrix outer body to facilitate release of the hydrophilic core.

## **2.6. Microbicide candidates**

It is not the remit of this article to provide a comprehensive review of the dozens of HIV microbicide candidates that have been evaluated over the years. Instead, the focus here is on those molecules that either have previously been tested or are being actively developed in VR formulations. A short overview of non-specific microbicides is presented first, before a more comprehensive review of ARV-based microbicides, and particularly the lead candidate ARVs DPV and TFV.

### **2.6.1. Non-antiretroviral microbicides**

First generation microbicide candidates tended to be non-antiretroviral compounds that had broad-spectrum activity against HIV and other sexually transmitted infections (STIs) (Table 3). These non-specific microbicides covered several modes of action, often not specific to the HIV life cycle and including surface active detergents and surfactants that could destroy or disrupt the viral membrane, pH acidifiers that could maintain the protective pH of the vagina and long chain

polyanionic compounds that could non-specifically inhibit viral fusion, attachment and entry into the host cells [103–107].

Although the majority of broad-spectrum microbicide candidates (e.g. Savvy, BufferGel, cellulose sulfate) were formulated in vaginal gels and creams, several VR formulations have also been evaluated (Table 3). In 2003, a silicone elastomer matrix-type VR releasing nonoxynol-9 was reported [18]. Although N-9 had shown promising antiviral activity against HIV-1 and other sexually transmitted infections *in vitro* [107–109], development of N9 microbicide products was halted following the discovery that it failed to provide protection against HIV-1 during clinical trials and that repeated exposure to the spermicidal compound actually damaged the vaginal epithelium and increased the risk of HIV-1 acquisition [19,110–112]. ZA has shown early promise as a broad-spectrum microbicide against HIV-1 and herpes simplex virus 2 (HSV-2) [113–115], despite an unknown mechanism of action.

Another non-specific microbicide being developed as a ring formulation is Boc-LBA, a betulonic acid derivative with anti-HIV activity *in vitro* [66]. This multi-step entry and fusion inhibitor was first formulated in a bio-soluble acacia gum reservoir-type VR (Table 3) [26,66]. More recently a nanoporous hydrophilic hydrogel-based combination ring product (Biorings<sup>TM</sup>) containing Boc-LBA in combination with ferrous gluconate (a non-hormonal contraceptive), ascorbic acid, polyamino-polycarboxylic acid mixtures and the nucleotide reverse transcriptase inhibitor (NRTI) TFV is undergoing early preclinical development by BioRing LLC [67].

Griffithsin (GRFT), a naturally occurring lectin found in red algae (*Griffithsia sp.*) is also undergoing early preclinical evaluation in a VR device. GRFT has shown potent, broad-spectrum

antiviral activity as a non-specific entry inhibitor against HIV-1, HIV-2 and other STIs including HSV-2 *in vitro* warranting further investigation as a topical microbicide [116–119]. Studies have shown that GRFT, a carbohydrate binding agent, binds to mannose rich glycans on viral envelope glycoproteins of HIV and HSV-2, coating the virus surface and thereby preventing/inhibiting penetration of host cells [117,120].

The high profile clinical failures of many non-ARV microbicides [121–123] has directed the microbicide field, and VR development specifically, towards use of potent ARVs that act specifically against the HIV life cycle. However, concerns over the development of ARV resistant strains mean that there may still be a place for non-specific, broad-spectrum microbicidal agents that are safe and effective against HIV and a range of sexually transmitted infections.

### **2.6.2. Antiretroviral microbicides**

A large number of both approved and experimental ARV drugs have been evaluated for formulation in VR devices (Table 4). The two lead candidate microbicides – TFV and DPV – are now discussed in detail.

Tenofovir ((([(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy)methyl)phosphonic acid, also known as PMPA, Table 4) and its oral prodrug form TDF (marketed as *Viread*, Table 4) are nucleotide analogue antiviral drugs made by Gilead Sciences Inc. and commonly used in the treatment of HIV infection. Consistent with other hydrophilic, negatively charged, acyclic phosphonate nucleotide analogues, TFV suffers poor oral bioavailability. TDF, however, in which the negative charges of its phosphonic acid groups are masked by phosphodiester

modification, is significantly more lipophilic resulting in greatly enhanced oral bioavailability [124–127]. Following absorption, TDF rapidly undergoes esterase hydrolysis to TFV, which is then metabolized intracellularly to its active anabolite tenofovir diphosphate, a competitive inhibitor of HIV-1 reverse transcriptase that interferes with and terminates DNA replication.

In 2006, Gilead Sciences granted a co-exclusive, royalty-free license to CONRAD and the IPM to develop TFV vaginal formulations for use by women in developing countries to prevent HIV infection. Since then, a large number of journal papers have been published describing either TFV or TDF formulated for vaginal application, including gels [128–144], tablets [145–147], nanoparticles [148–150] and VRs [43,61–63,68,83,86–89,91,151,152].

The hydrophilic nature of both TFV and TDF results in poor release characteristics from traditional permeation-controlled VR formulations fabricated from silicone elastomer or EVA, a consequence of the limited solubility of the TFV and TDF in these polymeric materials. Therefore, many of the VR strategies pursued for these drugs have involved use of novel ring designs and/or alternative polymer systems that overcome this permeation obstacle. The various ring types reported for delivery of TFV and TDF are summarized in Table 5. ‘Pod VRs’ comprise one or more drug-loaded pods embedded within a non-medicated silicone elastomer ring body containing delivery channels sited adjacent to each pod (Figure 2). The pods themselves are effectively small, compressed, solid tablets coated with a semi-permeable polymer (such as polylactic acid) to offer osmotic control [86]. By adjusting the number of pods and the width of the delivery channels, VRs can be fabricated containing multiple drug compounds with independent control of release rate. These pod rings have been widely studied for controlled release of TFV and TDF, either alone [88,89] or in combination with other ARV [91], antiviral

[86,89] and contraceptive drugs [91]. Typically, pod rings offer near constant drug release rates *in vitro* and maintain constant levels in the relevant biological compartments (cervicovaginal fluid, vaginal tissue and blood plasma) during the ring use period. In a 28-day comparative pharmacokinetic study in sheep, pod rings containing TDF produced drug tissue levels 86-fold higher than similar ring containing TFV, despite similar concentrations of each drug reported in cervicovaginal lavage [88]. A similar pharmacokinetic study in pig-tailed macaques demonstrated that TFV concentrations in vaginal lavage and tissue could be modulated by modification of the pod ring design by adjusting the size of the TFV reservoir and/or the width of the delivery channel [87]. The ability of the pod VR design to simultaneously deliver multiple drug compounds makes it an interesting platform for development of a multipurpose technology (MPT) VR, as exemplified by a pharmacokinetic study in sheep demonstrating steady state release of five – TFV, NVP, SQV, ETN and E2 – from a single pod-type ring device [91].

An alternative approach that has been used successfully to provide sustained vaginal delivery of TFV or TDF involves VR devices manufactured, at least in part, using hydrophilic thermoplastic polyurethanes (TPUs). The ability of these polymers to swell in the presence of aqueous liquids (including *in vitro* release media and presumably vaginal fluid) offers an alternative release mechanism to the permeation control offered by rings fabricated from hydrophobic silicone elastomer and EVA materials. Equilibrium water absorption values for TPUs can range from 20–900% depending on selection of the polymer grade. For VR fabrication, water absorption capacities at the lower end of the range are used [43,83], since excessive swelling *in vivo* would likely be problematic from the perspectives of both ring expansion / mechanical pressure and vaginal fluid uptake. Rings containing two different matrix-type TPU segments, one hydrophilic and the other hydrophobic, have been reported for co-delivery of TFV and DPV [43]. As

expected with matrix-type configurations, the amount of drug released from these rings decreased with time. By incorporating TFV powder or a TFV+glycerol+water mixture into the lumen of extruded TPU tubing and then joining the ends of the tubing to form a reservoir-type ring device, zero-order TFV release kinetics were achieved [83]. Release rates were greater for rings comprising the TFV+glycerol+water mixture, and the TFV release rate increased with equilibrium swelling value of the hydrophilic TPU. In a sheep PK study, rings fabricated from the 35% w/w swelling TPU and containing the TFV+glycerol+water mixture provided maintenance of TFV vaginal fluid concentrations close to  $10^6$  ng/g over the 90-day study period [83]. By comparison, the control 1% w/w TFV gel administered once daily showed TFV vaginal fluid levels steadily declining from  $10^6$  to  $10^4$  ng/g over a 28-day period. A similar ring design containing TDF (rather than TFV) in combination with sodium chloride as an osmotic agent offered protection against repeated vaginal challenge with simian human immunodeficiency virus 162p3 (SHIV162p3) in pig-tailed macaques over 28 days [63]. TFV levels in vaginal secretions and tissue remained consistent for 6 months with no adverse safety concerns [152].

TFV vaginal gel has previously shown a 39% reduction in HIV and an unanticipated 51% reduction in HSV-2 acquisition when used by women [13]. This HSV activity has also been demonstrated in *in vitro* cell and explant models for a TPU VR containing TDF [62], further supporting the concept of a MPT ring. TFV, in the form of a glycerol paste, has also been successfully combined with the contraceptive progestin LNG in a segmented dual-reservoir TPU VR offering continuous release of both drugs over 90 days [61].

Dapivirine (DPV), also known as 4-[[4-[(2,4,6-trimethylphenyl)amino]-2-pyrimidinyl]amino]benzonitrile and referred to in the early literature as TMC120, is an

experimental ARV drug that acts against HIV by inhibiting the reverse-transcriptase enzyme. Like many ARV HIV microbicides, DPV was originally developed – by Janssen Research and Development (formerly Tibotec Pharmaceuticals Ltd.), a subsidiary of Johnson & Johnson – as an oral ARV compound for treatment of HIV/AIDS. However, DPV showed such poor oral bioavailability in early stage clinical studies (due to its extremely low aqueous solubility) that this treatment option was abandoned. The compound was subsequently repurposed for vaginal application in 2004 when Tibotec granted IPM a non-exclusive, royalty-free license to develop DPV as a microbicide for use in low/middle income countries. In 2014, Janssen granted IPM exclusive worldwide rights to DPV. During this time, DPV has been extensively tested in a wide range of vaginal formulations, including gels, films, rings, freeze-dried matrices, nanoparticles, capsules, tablets and rings. IPM has completed numerous Phase I/II clinical trials of the compound in Africa, Europe and the United States (Table 6), all of which have demonstrated good safety, tolerance, user acceptability and pharmacokinetic profile. In response to concerns over poor user adherence to gel products and the preference for a single device offering sustained vaginal delivery of ARV compounds over extended time periods, IPM have now prioritized development of their DPV-releasing VR [153].

Proof-of-concept for a DPV-releasing VR was first demonstrated in *in vitro* studies that reported continuous, zero-order release from core-type (also known as reservoir-type, Figure 2), silicone elastomer, VRs over 71 days [20]. Based on upper limits for the volumes of cervicovaginal fluid and semen, and assuming *in vivo* release rate matched *in vitro* release, the 136 µg/day release rate was calculated to be capable of maintaining vaginal concentrations of DPV several orders of magnitude in excess of reported HIV inhibitory concentrations. Subsequent Phase I clinical studies conducted in Belgium assessed the safety and PK of a matrix-type (25 mg DPV loading)



and two different core-type (25 mg and 200 mg DPV loadings) silicone elastomer VRs [56,85]. The 25 mg matrix-type ring, in which the solid crystalline drug is dispersed throughout the entire volume of the device, produced higher concentrations of DPV in vaginal fluid, vaginal tissue and blood plasma compared with the core-type rings, reflecting the ready availability of drug at the ring surface. Both the core-type and matrix rings were safe and well tolerated and delivered DPV to the vaginal region for 28 days at concentrations over 4 logs greater than the EC<sub>50</sub> for wild-type HIV-1 (LAI) in MT4 cells (0.3 ng/mL) [154,155]. Importantly, systemic exposure of DPV with all ring formulations was deemed sufficiently low to alleviate concerns concerning the emergence of resistance strains of HIV.

Early DPV ring prototypes were fabricated using condensation-cure silicone elastomer systems [20,26,56,85,153]. The curing reaction associated with these materials produces a volatile alcohol by-product that detrimentally affected DPV distribution within the ring and its release after storage. As a result, the current version of the DPV-releasing VR, a 28-day matrix-type device containing 25 mg micronised DPV (Ring-004), is fabricated using an addition-cure silicone elastomer that produces no cure by-product. Compared with core-type rings (Figure 2), the simplicity of the matrix design of Ring-004 ensures ease of manufacture, low cost of manufacture, and higher pharmacokinetic exposure. A recent safety and pharmacokinetic study in women testing consecutive use of multiple 004 rings for up to 57 days reported detectable DPV concentrations in vaginal fluid and plasma within 4 hr after ring insertion, indicating rapid release and absorption of DPV [76].

With a view to expanding options for testing human-sized VR formulations in animal models, Holt et al. recently reported safety and pharmacokinetic evaluation of the DPV Ring-004 in

Suffolk cross sheep [156]. DPV plasma and vaginal fluid levels were lower than those measured in previous ring clinical studies [56,76,85,157]. DPV was also detected remotely in the neighboring rectal compartment, as reported previously with vaginal administration of the experimental entry inhibitor CMPD167 using aqueous gels and VRs [158].

IPM and clinical trial partner the Microbicide Trials Network (MTN) are currently conducting two Phase III long-term safety and efficacy studies of the monthly DPV ring as part of IPM's DPV Ring Licensure Program, with efficacy results expected as soon as early 2016 (Table 6). The Ring Study, started in April 2012 and conducted by IPM, enrolled 1,959 HIV-negative women aged 18 to 45 across seven research centers in South Africa and Uganda. The ASPIRE study, started in August 2012 and conducted by MTN, enrolled 2,629 HIV-negative women aged 18 to 45 across 15 sites in Malawi, Uganda, South Africa and Zimbabwe. In both trials, women were randomly assigned to use the monthly Ring-004 or placebo rings for at least one year. Results of both studies will be reported early 2016.

## **2.7. Combination microbicide and multipurpose prevention technology (MPT) rings**

HAART for treatment of HIV/AIDS involves the use of ARV combinations. By using drugs from different therapeutic classes and with different mechanisms of action, the virus is targeted at multiple stages of the infection/replication cycle, which can increase the breadth of activity and reduce the propensity for emergence of resistant viral strains [159–162]. It is rational to extend this combination strategy to ARV-based vaginal microbicides [163,164]. A combination of emtricitabine (a nucleoside reverse transcriptase inhibitor) and TDF (a nucleotide analogue reverse transcriptase inhibitor) administered orally has already been shown to confer HIV

protection in men who have sex with men [165] and the same combination was investigated as part of the VOICE trial [166].

A number of combination microbicide VRs are in the early stages of preclinical / clinical development. Following on the heels of the DPV Ring-004 are second-generation formulations containing DPV in combination with maraviroc (MVC) [80,157], darunavir (DRV) [81] and TFV [43]. MVC is an entry inhibitor ARV that binds the CCR5 co-receptor and prevents the cell entry of the most frequently transmitted HIV-1 strains [167–170]. It is considered a highly promising microbicide candidate because of its activity against HIV strains resistant to other ARVs and its use as a component of current HAART regimes. Aqueous vaginal gel formulations of MVC have previously been shown to prevent the vaginal transmission of SHIV-162P3 to macaques [171,172], and subsequent PK testing of the gels in macaques has helped define the local concentrations required for protection [158,173]. The first report of a MVC-only VR formulation demonstrated that pretreatment of macaques with Depo-Provera (a subdermal injectable contraceptive) significantly modified biodistribution of the drug [96]. Following a 2008 licensing agreement with Pfizer (now ViiV Healthcare), IPM is developing MVC as a microbicide, initially as a combination with DPV in a matrix-type ring device. Results from the MTN-013/IPM026 clinical study (a multisite PK/pharmacodynamics (PD) study among 48 women of silicone elastomer VR containing 25 mg DPV, 100 mg MVC, both DPV and maraviroc, or placebo; Table 6) showed that (i) MVC vaginal fluid concentrations in both the maraviroc-only arm and the combination arm were 2–10 times lower than DPV levels (despite the higher initial drug loading), (ii) cervical tissue levels of MVC were mostly below the limit of quantification, and (iii) no *in vitro* HIV inhibitory activity was observed with the maraviroc cervical tissue samples [157]. It was concluded that MVC was not released from the rings in sufficient quantities to

provide cervicovaginal concentrations capable of providing protection against HIV transmission. Previous *in vitro* testing of the same combination ring device showed that the quantities of DPV and MVC released from a 25 mg DPV + 100 mg MVC combination matrix-type silicone elastomer ring were similar [80]. Therefore, the poor release of MVC from the same ring formulation in the clinical setting is most likely due to physiological constraints placed upon MVC in the *in vivo* environment. These could include poor solubility in vaginal fluid, poor stability in vaginal fluid, poor tissue absorption, and/or rapid elimination from the tissue.

DRV is a second-generation PI used in combination with other ARVs in the treatment of HIV infection. PIs inhibit the HIV protease enzyme required to produce mature infectious virus particles by cleaving structural proteins and enzymes from their precursors. Their high potency within HAART regimens and the relatively high genetic barrier to the emergence of resistant HIV strains (compared with other ARVs) suggest they have good potential as microbicides, administered alone or in combination with other ARVs [174,175]. Preclinical development, including testing of pharmacokinetics in macaques, has recently been reported for matrix-type silicone elastomer VRs containing various loadings of DPV and DRV [81]. Serum and vaginal concentrations of both DPV and DRV in macaques during 28-day ring placement were measured within the same general range to those reported previously for DPV-only rings in women [56,76,85,157]. Based on the results of this study, the potential of PIs as vaginal microbicides, either alone or in combination, warrants further investigation.

VRs composed of biosoluble acacia gum or a non-biodegradable hydrogel of 2-hydroxyethyl methacrylate and sodium methacrylate have previously been assessed for formulation of microbicide combinations, selected from DPV, TFV, AZT and Boc-LBA [66]. A potential issue

with these gum and hydrogel matrices for ring fabrication is their propensity to absorb aqueous fluids and swell, which could be problematic *in vivo*. These ring formulations are not being actively developed.

The combination microbicide VRs discussed so far have been limited to two ARV drugs incorporated into the same compartment within the ring device, a strategy previously exploited with the contraceptive ring Nuvaring in which etonogestrel and ethinyl estradiol are located within the same core. However, this simple and relatively inexpensive approach to incorporating multiple drug compounds within a ring also introduces challenges, including increased potential for drug-drug interactions and reduced ability to independently control the release of each drug. A formulation strategy to overcome these challenges involves the fabrication of ring devices having multiple separate compartments, each compartment containing a single drug active. Several variations on this formulation approach have been reported. Dual-segment rings comprising DPV incorporated into a hydrophobic polyurethane segment and TFV incorporated into a hydrophilic polyurethane segment (Figure 5) showed good drug stability and *in vitro* release properties [43]. This approach is particularly useful for microbicide molecules having contrasting hydrophilic/hydrophobic character.

Despite the fact that a safe and effective vaginal microbicide product has yet to reach market, there is already considerable interest and early-stage development activity around next-generation products that combine HIV prevention with contraception and/or prevention/treatment of other sexually transmitted infections (STIs) and reproductive tract infections. Formulation strategies for multipurpose prevention technologies (MPTs) are generally based upon the extensive range of product types available within both the mature contraceptive market and the emerging HIV

microbicide pipeline [48]. Many of the MPT products currently undergoing development have prioritised use of LNG as the contraceptive hormone component, based on its historical record of safety and effectiveness [46,176,177]. Both DPV and TFV are being developed as MPT rings in combination with LNG [61,153]. Clark et al., describe a segmented dual-reservoir polyurethane VR (Figure 1G) that delivered the TFV and LNG continuously for 90 days in a rabbit pharmacokinetic model [61]. TFV was incorporated into a hydrophilic polyetherurethane reservoir segment in the form of a glycerol paste, while the levonorgestrel was located in a separate polyetherurethane reservoir segment. A DPV+LNG VR is also in development, based on a similar silicone elastomer matrix-type design to that of the dapvirine-only VR [153].

A number of MPT VR prototypes containing ZA in combination with the ARV agent MIV-150, the linear sulfated polysaccharide CG and the contraceptive steroid LNG are being actively developed by The Population Council (Table 3). A 2014 study by Ugaonkar et al. reported that sustained *in vitro* release of ZA from MIV-150/ZA/CG and MIV-150/ZA/CG/LNG combination core-type EVA VRs could be achieved for up to 90 days thus offering the potential for protection from HIV-1, HSV-2 and unwanted pregnancy from a single ring device [60]. Results from macaque efficacy and pharmacokinetic (PK) studies have also been promising, indicating that the ZA combination VR devices are capable of providing protection from SHIV-RT and reducing viral shedding of HSV-2 [60].

### **3. Challenges moving forward**

#### **3.1. Formulation and delivery of biomacromolecular microbicides**

Small-molecule ARVs are currently the major focus of the microbicide field. However, there is some interest in the use of biomacromolecular compounds as vaginal microbicides, including

proteins (cyanovirin-N, GRFT, 5P12-RANTES), peptides (T-1249, PIE12 trimer, rectrocyclin RC-101), monoclonal antibodies (mAbs) (b12, 2F5, 4E10, 2G12, VRCO1) and nucleic acids (DNA, short interfering RNA (siRNA)). Many of these biopharmaceuticals agents can inhibit transmission of HIV and other STIs by either directly targeting the free virus or by blocking the host cell receptors [178–180].

Broadly neutralizing mAbs such as b12, 2F5, 4E10 and 2G12 [93,181–185] have shown promise due to their high potency, excellent safety profile and their unique ability to be both specific whilst having a broad spectrum of action when combined in a multi-antibody formulation [178,180]. MABGEL1, a monoclonal antibody gel containing 2F5, 4E10 and 2G12 developed by the European Microbicides Programme (EMPRO) was the first reported mAb vaginal product to undergo Phase I pharmacokinetic and safety testing. The study demonstrated that daily application of up to 50 mg of each Ab was safe over a 12 day period and was able to achieve concentrations with the potential to block HIV transmission. However, stability of the mAbs was a significant issue for these gel formulations [185]. Until recently challenges regarding production costs, production capacity, quality control and safety of biological therapeutics has prevented large-scale development and evaluation of mAbs in a microbicial formulation [178]. However, recent advances in the production of mAbs in plants such as *Nicotiana benthamiana* has provided the potential for safe, rapid, cost effective production of N-produced recombinant human monoclonal antibodies (N-mAbs) [186].

The first antibody-releasing VR was reported in 1992 [187]. These proof-of-concept VR devices formulated using lyophilized antibody particles of bovine serum albumin (BSA) and anti-human chorionic gonadotropin (anti-hCG) in thermoplastic EVA demonstrated sustained Ab release for

up to 30 days and prevented HSV-2 transmission in mice, thus paving the way for future Ab-releasing ring studies [188,189]. In 2011 Morrow et al., reported that a rod-insert VR device was capable of releasing the mAb 2F5 [93]. The ring comprised a silicone elastomer ring body into which multiple 2F5-loaded lyophilized hydropropylmethylcellulose (HPMC) gel inserts could be placed. *In vitro* release testing demonstrated that the rod-insert device was capable of delivering over 1 mg of 2F5 for a period of up to 100 hr dependent on the lyophilized gel insert formulation. These VR devices provided the capability to deliver temperature-sensitive biologically-based microbicides as production of the lyophilized gel inserts did not involve the use of high temperatures normally associated with polymer ring manufacture [16].

Currently, several antibody-containing VRs are undergoing early preclinical development. In a recently reported study by Gunawardana et al., a novel pod-type platinum-catalysed silicone elastomer VR demonstrated sustained *in vitro* delivery of ovine IgG (ov-IgG), a model IgG human antibody, over a period of 14 days further confirming that a VR device has the potential to provide sustained effective release of antibody-based microbicides [86,92]. Mapp66 is a novel multi-antibody microbicide currently under investigation by Integrated Preclinical/Clinical Program for HIV Topical Microbicides (IPCP-HTM) in conjunction with Mapp Biopharmaceutical Inc. [190]. Mapp66 contains a triple combination of N-mAbs (4E10-N, VRCO1-N and HSV8-N) that have the potential to neutralize a range of HIV isolates and prevent sexually transmitted HSV-2 infection. Early studies suggest that a mapp66 VR device utilizing the Versaring pod-insert technology developed by Auritec Pharmaceutical and Oak Crest Institute of Science [86] is capable of efficient intravaginal release of N-Mabs [191].



In addition to monoclonal antibodies, sustained delivery of Llama heavy-chain antibody fragments (VHH) have been reported from a rod-type ring device [192]. Similar to the rod-insert rings reported by Morrow et al. [93], these VR devices manufactured using silicone elastomers were capable of holding multiple HPMC compressed or lyophilized gel antibody tablets. *In vitro* release testing demonstrated effective release of the highly potent HIV-1 entry inhibitor (VHH A12) over a 7-day period in concentrations sufficient to offer protection in the vaginal environment [192].

As discussed earlier in this review (see Section 2.6.1.) GRFT, a naturally occurring algal protein is also undergoing early preclinical evaluation as a potential virus entry inhibitor against multiple STIs including HIV-1, HIV-2 and HSV-2. VR devices containing GRFT in combination with CG or MIV-150 are currently under evaluation [119,193].

Whilst these biopharmaceuticals have shown early promise as microbicidal candidates their high production costs, stability and formulation issues remain major obstacles for their successful development as effective microbicidal products.

### **3.2. Manufacturing issues and scale up**

A potential disadvantage of silicone elastomer VRs is the increased complexity in scaling the elastomer/API mixing processes. Most silicone elastomer VR projects use DAC mixers to disperse API into the elastomer base. However, scalability of this equipment limits batch sizes to 5 kg, thereby requiring totally new classes of mixers to be trialed and validated for anything other than early clinical testing. Thermoplastic extrusion processes on the other hand are generally scalable provided that the screw geometry and length to diameter ratio of the extruder are

appropriately matched. Also, given the high output capabilities of hot melt extrusion equipment, it is feasible to use the same equipment for early clinical trial product manufacture through to commercial scale production by simply increasing manufacturing duration. A particular disadvantage associated with thermoplastic extrusion as a manufacturing technique to produce VRs is the requirement to cut, bend and weld the extrudate ends to form a full ring; this process is complex to automate and ultimately the rate limiting step in the production output. These issues are not present for thermoplastic or silicone elastomer rings manufactured via injection molding.

Suppliers of injection molding and extrusion equipment often have limited experience of the pharmaceutical industry and hence the requirements of cGMP and stringent quality systems that must be employed. Significant investment in partnerships between original equipment manufacturers and pharmaceutical stakeholders has been necessary to commission suitable equipment. As these manufacturing techniques become more widely adopted by the pharmaceutical industry, equipment that is capable of fulfilling cGMP requirements should be more readily available across the injection molding and extrusion equipment supply sectors.

Multi-cavity injection mold tooling for production of high volumes of rings per cycle require significant detail and technical expertise to ensure that conditions such as pressure and temperature are uniformly experienced for each ring cavity. In particular, silicone elastomer mold tooling is highly specialized with only a handful of companies worldwide with the expertise to machine tools with the significantly higher tolerances required compared to thermoplastic tools. Limited choice of contract manufacturing organizations (CMO) with the expertise and capability to manufacture either thermoplastic or thermosetting-based VRs has also slowed the progress of development of microbicide VR products.

1119  
1120 Methods for determining the assay value (drug content) of VRs can be time consuming and  
1121 costly. A method for Process Analytical Testing (PAT) has been proposed using Raman  
1122 spectroscopy for the 25 mg DPV ring currently under development [194,195]. This method  
1123 rotates a manufactured ring whilst the Raman spot is focused on a fixed point providing wide  
1124 area illumination and the results were correlated to provide a prediction of content assay values  
1125 for the ring with good levels of accuracy.

### 1126 1127 **3.3. Cost**

1128 Since the inception of the vaginal microbicide concept in 1990 [12], the cost factor has been  
1129 uppermost in the minds of developers. The impact of the HIV pandemic is greatest in Sub-  
1130 Saharan Africa and Asia/Pacific region, where 24.7 million and 4.8 million respectively are  
1131 currently living with HIV/AIDS; Sub-Saharan Africa alone accounts for almost 70% of the  
1132 global total of new HIV infections. Many of the countries within these regions have gross  
1133 domestic product per capita values significantly less than \$1000, with major implications for the  
1134 availability and quality of healthcare provision. In order to gain widespread use, HIV microbicide  
1135 products must be affordable to at-risk populations. As with all pharmaceutical products,  
1136 manufacturing costs will comprise a very substantial part of the total cost structure of a  
1137 microbicide product, including the costs of the active pharmaceutical ingredients, formulation  
1138 excipients and product packaging. For the 1% TFV gel tested in the CAPRISA 004 trial,  
1139 manufacturing costs were reported as US\$ 0.50 per dose, a significant proportion of which was  
1140 for provision of the plastic applicator [196]. By comparison, microbicidal VRs will be much  
1141 more costly to manufacture, due to increased drug loadings, complexity of product design,  
1142 advanced manufacturing processes and the use of relatively expensive excipients. However,

unlike gels, for which a new dose needs to be applied either daily or before every act of intercourse (depending on the prescribed regimen), microbicide-releasing VRs currently in development are intended to be worn continuously for at least 28 days. This longer duration of use compared with gels will compensate to some degree for the increased costs of ring manufacture. Assuming a fixed manufacturing cost per ring device, extending the duration of ring use will result in a proportional lowering of the daily cost of use. IPM is developing 60 and 90-day versions of their 28-day DPV-releasing ring [153]. Advocates are working with researchers and policy makers to make sure that any approved microbicide will be as affordable and accessible as possible. For example, efforts are already underway to ensure that manufacturing costs of the DPV ring are kept as low as possible.

### **3.4. Acceptability and Adherence**

Numerous studies have reported high user acceptability of VRs for contraception and estrogen replacement therapy [29,30,33–35,39,197–202]. Of particular significance is the strong preference for rings over semi-solid systems [34,197] which should hopefully extend to vaginal microbicide products, since high levels of user acceptability/satisfaction generally correlate with user adherence.

Medication adherence is defined as the extent to which users/patients take their medications as prescribed. An estimated 20% to 50% of patients do not take their medications as prescribed and are said to be non-adherent or non-compliant with therapy [203,204]. Medication non-adherence is a major and growing concern for many current drug therapies, including HAART for the treatment of HIV infection [205] and statin medication for chronic coronary artery disease [206]. User adherence to vaginal microbicide products in late-stage clinical studies has proved

problematic, particularly for regimens that require regular daily application (i.e. once-daily products) or require timing of application close to coitus (i.e. coitally-dependent products) [207–209]. The most widely cited example is that for the Phase IIb CAPRISA trial of vaginal TFV gel, in which HIV acquisition was reduced by an overall estimated 39% [13,210–212]. However, adherence estimates based on vaginal applicator returns indicated that HIV incidence was 54, 38 and 28% lower in the TFV gel arm for high, intermediate and low adherers, respectively, demonstrating unequivocally that high adherence is key to microbicide effectiveness. In fact, it has primarily been the growing concern over lack of user adherence to gel-based microbicides in clinical studies that has driven the prioritization of ARV-releasing VR products [213,166,214].

It has long been assumed that use of sustained or controlled release delivery systems for vaginal administration of microbicides to prevent infection with HIV will lead to increased microbicide product adherence, acceptability and efficacy compared with more conventional, coitally-dependent, vaginal formulations by simplifying use instructions and requiring less user behavior [15,16,56,215]. Indeed, based on adherence data from other clinical indications [204,216,217], including hormonal contraception for which long-acting depot injections, sub-dermal implants, transdermal patches and VRs are available [218,219], the case for sustained/controlled release of HIV microbicides is generally well made and widely accepted. Previous studies have reported high levels of user adherence to VRs for non-microbicide clinical indications. For example, in a 3-month study comparing adherence to the contraceptive VR Nuvaring and a daily low-dose oral contraceptive pill, ring users were more likely to report perfect use [220]. Surprisingly, given the acknowledged importance of adherence, only a very limited number of studies have directly addressed the topic of adherence to microbicide VRs [221–223].

One of the major challenges for the HIV microbicide field is the accurate (and preferably quantitative) measurement of adherence in late stage clinical trials [207,215,224]. Generally, methods for measuring adherence can be divided into two distinct categories. Direct measures of adherence, also referred to as “biomarkers”, are substances or effects whose presence or absence indicates that a biological or pharmacological process has occurred in response to a drug [207]. Indirect measures of adherence comprise two major sub-categories: “objective measures” and “self-report measures”, both reliant on the observations or reports of clinicians, trial participants, or others [207,225]. Self-reporting tends to overestimate adherence behavior compared with other assessment methods and generally has high specificity but low sensitivity [225]. Some of the methods previously reported for assessing adherence to microbicides are specific to a particular product type. For example, several advanced vaginal gel applicators have been developed, either containing a dye that changes color upon exposure to mucin or that record the date and time that the syringe piston is depressed into the applicator barrel [226]. Both Phase III clinical studies for the DPV ring – The Ring Study and APSIRE – will attempt to measure women’s adherence to the ring by measuring concentrations of DPV in blood and vaginal fluid and testing the residual DPV content in rings after 28-day use.

The recording of vaginal temperature offers an alternative and interesting biomarker option for monitoring adherence to microbicide-releasing VRs. Boyd et al. recently reported the testing in macaques of a vaginally-administered silicone elastomer device fitted with a miniature, battery-operated, temperature logger [97]. The device responded quickly and accurately to vaginal removal and insertion, and produced a regular diurnal temperature pattern comprising higher temperatures during daytime activity and lower temperatures during nighttime inactivity (matching the diurnal cycle observed in a woman’s basal body temperature). Ring devices fitted

with temperature loggers could be used to directly monitor user adherence as part of late-stage clinical testing.

### **3.5. Correlating *in vitro* release with *in vivo* pharmacokinetics**

Development of *in vitro-in vivo* correlations (IVIVC) for complex, non-oral, extended release products is a long-term aim of many pharmaceutical development programs [227]. The overall aim is to reduce the regulatory burden associated with certain pre- and post-approval changes. For example, manufacturing process, equipment and site changes can be reduced in the presence of a Level A or point-to-point IVIVC. However, developing IVIVC for non-oral extended release products is extremely challenging due to the complex nature of the formulations and the difficulty in accurately mimicking the *in vivo* release process with an *in vitro* method [227]. These problems are magnified in the microbicide ring field due to the number of variables about which we have limited information and the fact that many of the biological factors will vary throughout the hormonal cycle. There is also an awareness of the need to define PK-PD relationships for microbicides. This is also beset by challenges due to the unique nature of the products [176].

Completed, current and pending clinical studies involving microbicide VRs are presented in Table 6. Relatively few candidate microbicide compounds have proceeded to clinical testing in a ring device. The current Phase III clinical trials of the DPV 25 mg VR should provide key values for the vaginal fluid, tissue and plasma concentrations seen on repeated use in a much larger sample of women than has been reported to date [56,75,76]. This information, coupled with knowledge of the seroconversion status of trial participants, will help establish the vaginal fluid and tissue concentrations necessary for protection with this microbicide in a clinical setting. More

generally, how well these data relate to *in vitro* IC<sub>50</sub> values may also prove informative, giving an indication of how close *in vitro* estimates of activity are to the clinical scenario. However, because of the large differences between candidate microbicides physicochemical properties and mechanisms of action, other microbicide compound will need to be assessed individually [176].

The PK of DPV released from IPM's reservoir-type Ring-002 and matrix-type Ring-003 have been compared [56,153]. (The ring designs and silicone elastomer type used in these rings are not the same as for the Ring-004 design currently being tested in Phase III [153].) The matrix ring led to increased vaginal fluid and plasma levels compared with reservoir ring, although inter-subject variability was significantly lower for the reservoir ring. Interestingly, the vaginal fluid concentration profiles measured did not reflect the differences typically observed *in vitro* with these rings. The other microbicide tested clinically from a ring device is ACV. A ring containing 64 mg ACV was found to provide comparable cervicovaginal lavage concentrations over 7 or 14 days use, to samples provided 2 hr after oral valaciclovir ingestion [90].

Several attempts have been made to correlate *in vitro* release with that observed in different animal models. A selection of published non-human studies involving microbicide releasing VRs are presented in Table 7. Overall, these studies have provided some evidence for the development of IVIVC in animal species but whether and how this will translate to humans is unclear. First attempts at IVIVCs have been published for TFV and ACV release from pod-insert rings into rabbits and sheep [89], and for double and triple combination microbicide release (TDF, emtricitabine and MVC) in macaques [228].



Several articles have reported a lack of correlation between the *in vitro* and *in vivo* release rates. For example, *in vitro* release rate of UC781 was much higher than that observed *in vivo*, presumably attributed to the exceptionally poor water solubility of UC781 [79,229]. It has been reported that non-sink *in vitro* conditions exhibiting partition-controlled release better predicted the total amount of experimental pyrimidinedione microbicides released from polyurethane VRs in pigtail macaques, whereas sink *in vitro* release conditions, exhibiting typical matrix-type kinetics, over predicted release [83]. *In vitro* release of MVC and CMPD167 from silicone elastomer rings into simulated vaginal fluid (SVF) was a relatively good predictor of the amount released in rhesus macaques *in vivo* [96]. Notably, this work also highlighted the differences observed *in vivo* with the use of depot medroxyprogesterone acetate (DMPA) pre-treatment and the impact this can have on measured absorption. Release of the more hydrophobic MC1220 from matrix-type silicone elastomer rings in macaques was somewhere between that measured *in vitro* into a mixture of equal parts of propan-2-ol and water or SVF [69]. Other researchers have found conflicting results between *in vitro* and *in vivo* testing in animal models. For example, the *in vivo* concentrations of MIV-150 in vaginal fluids were similar when the microbicide was released from both silicone and EVA rings, despite the EVA rings having a higher drug loading and showing higher *in vitro* release rates [77].

Recently deterministic models of vaginal distribution of drugs delivered from both gels and rings have been presented [230]. Methods used to determine vaginal drug permeation have also recently been reviewed [231]. Given the highly complex and variable nature of the vaginal environment and the relative simplicity of the currently used *in vitro* release rate tests, it may prove difficult to effectively correlate values from one to another. However, it may be possible to draw some broad inferences from a given release rate test in relation to available clinically tested

products, as attempted in Table 8 for vaginal fluid concentrations, plasma concentrations and *in vitro* release data of DPV during use of the 25 mg VR. Available data for the same ring tested in sheep are presented as are data for a smaller macaque sized ring with the same 25 mg loading, composed of similar but not identical type of silicone. This table is informative if merely to show the large range of values that may be seen between *in vitro* release rates and those measured in vaginal fluid at any time.

In the first instance, the primary aim of such correlations should be to link previously established *in vitro* release rates with consistently achieved protective vaginal fluid and tissue levels in the compartments of interest. It might then be possible to tie together *in vitro* release rate testing, clinical PK profiles and *ex vivo* assays including challenge assays to provide a more holistic picture of drug loadings and release profiles necessary to afford protection. However, all of the above *in vitro* tests will need to be benchmarked against clinical concentration and effectiveness data. The ultimate usefulness of IVIVC may only be seen when sufficient clinical data is available to allow such comparisons to be drawn.

#### **4. Conclusions**

The past ten years has witnessed unprecedented advances in vaginal ring technology for the delivery of drugs, driven almost exclusively by the development of practical, long-acting and user-friendly vaginal microbicide products for prevention of sexual transmission of HIV. Considerable innovation in the development of novel ring designs has emerged in attempts to achieve clinically effective release rates for microbicide candidates that often possess very different physicochemical properties from the small molecular weight hydrophobic steroid molecules for which the original vaginal ring devices were first described back in the 1970s. The

1310 future of vaginal microbicide VRs will likely depend on the outcome of ongoing clinical studies  
1311 testing dapvirine and tenofovir-releasing rings. In particular, success of the monthly dapivirine  
1312 ring in two recently completed Phase III studies ('The Aspire Study' and 'The Ring Study') is  
1313 likely a prerequisite for the future viability of not only vaginal ring strategies for HIV prevention,  
1314 but for vaginal microbicides in general. If the key indicators for success are met – at least  
1315 moderate protection against HIV infection; long-term safety; ease of use; user acceptability; good  
1316 user adherence; global access – microbicide-releasing VRs are positioned to make a valuable  
1317 contribution in the fight against one of the greatest threats to women's health globally. Success  
1318 should also stimulate priority development of next-generation combination microbicide and MPT  
1319 VR products aimed at further enhancing protection, minimising development of resistant HIV  
1320 strains, and additionally offering contraception and protection against other STIs.

## REFERENCES

- [1] UNAIDS Factsheet 2015, (2015).  
[http://www.unaids.org/sites/default/files/media\\_asset/20150714\\_FS\\_MDG6\\_Report\\_en.pdf](http://www.unaids.org/sites/default/files/media_asset/20150714_FS_MDG6_Report_en.pdf)
- [2] D. Lema, A. Garcia, J.B. De Sanctis, HIV Vaccines: A Brief Overview, *Scand. J. Immunol.* 80 (2014) 1–11.
- [3] S.C. Weller, K. Davis-Beaty, Condom effectiveness in reducing heterosexual HIV transmission, in: S.C. Weller (Ed.), *Cochrane Database Syst. Rev.*, John Wiley & Sons, Ltd, Chichester, UK, 2002.
- [4] F.K. Giannou, C.G. Tsiara, G.K. Nikolopoulos, M. Talias, V. Benetou, M. Kantzanou, et al., Condom effectiveness in reducing heterosexual HIV transmission: a systematic review and meta-analysis of studies on HIV serodiscordant couples, *Expert Rev. Pharmacoecon. Outcomes Res.* 7167 (2015) 1–11.
- [5] R.A. Crosby, State of Condom Use in HIV Prevention Science and Practice, *Curr. HIV/AIDS Rep.* 10 (2013) 59–64.
- [6] T.T. Langen, Gender power imbalance on women's capacity to negotiate self-protection against HIV / AIDS in Botswana and South Africa, *Afr. Health Sci.* 5 (2005) 188–197.
- [7] S. Ray, J. van De Wijgert, P. Mason, F. Ndowa, C. Maposhere, Constraints faced by sex workers in use of female and male condoms for safer sex in urban zimbabwe., *J. Urban Health.* 78 (2001) 581–92.
- [8] C.S. Haignere, R. Gold, J. Maskovsky, J. Ambrosini, C.L. Rogers, E. Gollub, High-risk adolescents and female condoms: knowledge, attitudes, and use patterns., *J. Adolesc. Health.* 26 (2000) 392–8.
- [9] C. Joanis, M. Beksinska, C. Hart, K. Tweedy, J. Linda, J. Smit, Three new female condoms: which do South-African women prefer?, *Contraception.* 83 (2011) 248–54.
- [10] M. Beksinska, J. Smit, C. Joanis, W. Potter, New female condoms in the pipeline., *Reprod. Health Matters.* 20 (2012) 188–96.
- [11] A. Tseng, J. Seet, E.J. Phillips, The Evolution of Three Decades of Antiretroviral Therapy: Challenges, Triumphs and the Promise of the Future., *Br. J. Clin. Pharmacol.* (2014).
- [12] Z.A. Stein, HIV prevention: the need for methods women can use., *Am. J. Public Health.* 80 (1990) 460–2.
- [13] Q. Abdool Karim, S.S. Abdool Karim, J.A. Frohlich, A.C. Grobler, C. Baxter, L.E. Mansoor, et al., Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women., *Science.* 329 (2010) 1168–74.
- [14] R.K. Malcolm, A.D. Woolfson, Silicone intravaginal rings: A new delivery technology for HIV microbicides?, in: *Microbicides 2002*, Antwerp, 2002: p. 115, B–222.
- [15] J. Romano, R.K. Malcolm, S. Garg, L.C. Rohan, P.E. Kaptur, Microbicide delivery: formulation technologies and strategies., *Curr. Opin. HIV AIDS.* 3 (2008) 558–66.

- 1360 [16] R.K. Malcolm, K.-L. Edwards, P. Kiser, J. Romano, T.J. Smith, Advances in microbicide  
1361 vaginal rings., *Antiviral Res.* 88 Suppl 1 (2010) S30–9.
- 1362 [17] P.F. Kiser, T.J. Johnson, J.T. Clark, State of the art in intravaginal ring technology for  
1363 topical prophylaxis of HIV infection., *AIDS Rev.* 14 (2012) 62–77.
- 1364 [18] K. Malcolm, D. Woolfson, J. Russell, C. Andrews, In vitro release of nonoxynol-9 from  
1365 silicone matrix intravaginal rings., *J. Control. Release.* 91 (2003) 355–64.
- 1366 [19] L. Van Damme, G. Ramjee, M. Alary, B. Vuylsteke, V. Chandeying, H. Rees, et al.,  
1367 Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female  
1368 sex workers: a randomised controlled trial., *Lancet.* 360 (2002) 971–7.
- 1369 [20] R.K. Malcolm, A.D. Woolfson, C.F. Toner, R.J. Morrow, S.D. Mccullagh, Long-term,  
1370 controlled release of the HIV microbicide TMC120 from silicone elastomer vaginal rings.,  
1371 *J. Antimicrob. Chemother.* 56 (2005) 954–6.
- 1372 [21] G.W. Duncan, Medicated Devices and Methods, US3545439 A, 1970.
- 1373 [22] D.R. Mishell, M.E. Lumkin, Contraceptive effect of varying dosages of progestogen in  
1374 silastic vaginal rings., *Fertil. Steril.* 21 (1970) 99–103.
- 1375 [23] D.R. Mishell, M. Talas, A.F. Parlow, D.L. Moyer, Contraception by means of a silastic  
1376 vaginal ring impregnated with medroxyprogesterone acetate., *Am. J. Obstet. Gynecol.* 107  
1377 (1970) 100–7.
- 1378 [24] A.D. Woolfson, G.R.E. Elliott, C.A. Gilligan, C.M. Passmore, Design of an intravaginal  
1379 ring for the controlled delivery of 17b-estradiol as its 3-acetate ester, *J. Control. Release.*  
1380 61 (1999) 319–328.
- 1381 [25] K. Malcolm, D. Woolfson, J. Russell, P. Tallon, L. Mcauley, D. Craig, Influence of  
1382 silicone elastomer solubility and diffusivity on the in vitro release of drugs from  
1383 intravaginal rings., *J. Control. Release.* 90 (2003) 217–25.
- 1384 [26] R.K. Malcolm, S.M. Fetherston, C.F. McCoy, P. Boyd, I. Major, Vaginal rings for  
1385 delivery of HIV microbicides., *Int. J. Womens. Health.* 4 (2012) 595–605.
- 1386 [27] W. Bounds, A. Szarewski, D. Lowe, J. Guillebaud, Preliminary report of unexpected local  
1387 reactions to a progestogen-releasing contraceptive vaginal ring., *Eur. J. Obstet. Gynecol.*  
1388 *Reprod. Biol.* 48 (1993) 123–125.
- 1389 [28] E. Weisberg, I.S. Fraser, J. Baker, D. Archer, B.M. Landgren, S. Killick, et al., A  
1390 randomized comparison of the effects on vaginal and cervical epithelium of a placebo  
1391 vaginal ring with non-use of a ring., *Contraception.* 62 (2000) 83–9.
- 1392 [29] V. Brache, F. Alvarez-Sanchez, a Faundes, T. Jackanicz, D.R. Mishell, P. Lähteenmäki,  
1393 Progestin-only contraceptive rings., *Steroids.* 65 (2000) 687–91.
- 1394 [30] F.J. Roumen, D. Apter, T.M. Mulders, T.O. Dieben, Efficacy, tolerability and  
1395 acceptability of a novel contraceptive vaginal ring releasing etonogestrel and ethinyl  
1396 oestradiol., *Hum. Reprod.* 16 (2001) 469–75.
- 1397 [31] B. Harwood, D.R. Mishell, Jr., Contraceptive Vaginal Rings, *Semin. Reprod. Med.* 19  
1398 (2001) 381–390.

- 1399 [32] A.R. Thurman, M.R. Clark, J.A. Hurlburt, G.F. Doncel, Intravaginal rings as delivery  
1400 systems for microbicides and multipurpose prevention technologies., *Int. J. Womens.*  
1401 *Health.* 5 (2013) 695–708.
- 1402 [33] L.E. Nachtigall, Clinical trial of the estradiol vaginal ring in the U.S., *Maturitas.* 22 (1995)  
1403 S43–S47.
- 1404 [34] R. Barentsen, P.H. van de Weijer, J.H. Schram, Continuous low dose estradiol released  
1405 from a vaginal ring versus estriol vaginal cream for urogenital atrophy., *Eur. J. Obstet.*  
1406 *Gynecol. Reprod. Biol.* 71 (1997) 73–80.
- 1407 [35] A. Novák, C. de la Loge, L. Abetz, E. van der Meulen, The combined contraceptive  
1408 vaginal ring, NuvaRing®: an international study of user acceptability, *Contraception.* 67  
1409 (2003) 187–194.
- 1410 [36] A. van der Straten, E.T. Montgomery, H. Cheng, L. Wegner, G. Masenga, C. von  
1411 Mollendorf, et al., High acceptability of a vaginal ring intended as a microbicide delivery  
1412 method for HIV prevention in African women., *AIDS Behav.* 16 (2012) 1775–86.
- 1413 [37] F.G. Burton, W.E. Skiens, N.R. Gordon, J.T. Veal, D.R. Kalkwarf, G.W. Duncan,  
1414 Fabrication and testing of vaginal contraceptive devices designed for release of  
1415 prespecified dose levels of steroids., *Contraception.* 17 (1978) 221–230.
- 1416 [38] A.D. Woolfson, R.K. Malcolm, R.J. Morrow, C.F. Toner, S.D. McCullagh, Intravaginal  
1417 ring delivery of the reverse transcriptase inhibitor TMC 120 as an HIV microbicide., *Int. J.*  
1418 *Pharm.* 325 (2006) 82–9.
- 1419 [39] T.O.M. Dieben, F.J.M.E. Roumen, D. Apter, Efficacy, cycle control, and user  
1420 acceptability of a novel combined contraceptive vaginal ring., *Obstet. Gynecol.* 100 (2002)  
1421 585–93.
- 1422 [40] A. Loxley, M. Mitchnick, O. Okoh, J. McConnell, L. Goldman, C. Morgan, et al.,  
1423 Ethylene vinyl acetate intravaginal rings for the simultaneous delivery of the antiretroviral  
1424 UC781 and contraceptive levonorgestrel, *Drug Deliv. Transl. Res.* 1 (2011) 247–255.
- 1425 [41] C. McConville, I. Major, D.R. Friend, M.R. Clark, R.K. Malcolm, Development of a  
1426 UC781 releasing polyethylene vinyl acetate vaginal ring, *Drug Deliv. Transl. Res.* 2  
1427 (2012) 489–497.
- 1428 [42] K.M. Gupta, S.M. Pearce, A.E. Poursaid, H.A. Aliyar, P.A. Tresco, M.A. Mitchnik, et al.,  
1429 Polyurethane intravaginal ring for controlled delivery of dapivirine, a nonnucleoside  
1430 reverse transcriptase inhibitor of HIV-1., *J. Pharm. Sci.* 97 (2008) 4228–39.
- 1431 [43] T.J. Johnson, K.M. Gupta, J. Fabian, T.H. Albright, P.F. Kiser, Segmented polyurethane  
1432 intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine  
1433 and tenofovir., *Eur. J. Pharm. Sci.* 39 (2010) 203–12.
- 1434 [44] J.T. Clark, T.J. Johnson, M.R. Clark, J.S. Nebeker, J. Fabian, A.L. Tuitupou, et al.,  
1435 Quantitative evaluation of a hydrophilic matrix intravaginal ring for the sustained delivery  
1436 of tenofovir., *J. Control. Release.* 163 (2012) 240–8.
- 1437 [45] J. Balzarini, D. Schols, Combination of Antiretroviral Drugs as Microbicides, *Curr. HIV*  
1438 *Res.* 10 (2012) 53–60.

- 1439 [46] C. Woodsong, J. Holt, B. Devlin, Z. Rosenberg, Current Status of Multipurpose  
1440 Prevention Technology (MPT) Development, *Curr. Obstet. Gynecol. Rep.* 4 (2015) 43–52.
- 1441 [47] R.K. Malcolm, S.M. Fetherston, Delivering on MPTs : addressing the needs , rising to the  
1442 challenges and making the opportunities, *Contraception.* 88 (2013) 321–325.
- 1443 [48] R.K. Malcolm, P. Boyd, C.F. McCoy, D.J. Murphy, Beyond HIV microbicides:  
1444 multipurpose prevention technology products., *BJOG.* 121 Suppl (2014) 62–9.
- 1445 [49] C. Woodsong, P. Musara, a Chandipwisa, E. Montgomery, P. Alleman, M. Chirenje, et  
1446 al., Interest in multipurpose prevention of HIV and pregnancy: perspectives of women,  
1447 men, health professionals and community stakeholders in two vaginal gel studies in  
1448 southern Africa., *BJOG.* 121 Suppl (2014) 45–52.
- 1449 [50] Combination Products, (2009).
- 1450 [51] ISO 10993-1:2003. Biological evaluation of medical devices – Part 1: Evaluation and  
1451 testing, 2003.
- 1452 [52] F.W. van Der Weij, The action of tin compounds in condensation-type RTV silicone  
1453 rubbers, *Die Makromol. Chemie.* 181 (1980) 2541–2548.
- 1454 [53] R.K. Malcolm, S.D. McCullagh, a D. Woolfson, S.P. Gorman, D.S. Jones, J. Cuddy,  
1455 Controlled release of a model antibacterial drug from a novel self-lubricating silicone  
1456 biomaterial., *J. Control. Release.* 97 (2004) 313–20.
- 1457 [54] S.D. McCullagh, R.K. Malcolm, A.D. Woolfson, S.P. Gorman, D.S. Jones, J. Cuddy,  
1458 Release kinetics of oleyl alcohol from a self-lubricating silicone biomaterial, *J. Mater.*  
1459 *Chem.* 14 (2004) 1093.
- 1460 [55] A.D. Woolfson, R.K. Malcolm, R.J. Gallagher, Design of a silicone reservoir intravaginal  
1461 ring for the delivery of oxybutynin., *J. Control. Release.* 91 (2003) 465–76.
- 1462 [56] A. Nel, S. Smythe, K. Young, K. Malcolm, C. McCoy, Z. Rosenberg, et al., Safety and  
1463 pharmacokinetics of dapivirine delivery from matrix and reservoir intravaginal rings to  
1464 HIV-negative women., *J. Acquir. Immune Defic. Syndr.* 51 (2009) 416–23.
- 1465 [57] J. a H. van Laarhoven, M. a B. Kruff, H. Vromans, In vitro release properties of  
1466 etonogestrel and ethinyl estradiol from a contraceptive vaginal ring., *Int. J. Pharm.* 232  
1467 (2002) 163–73.
- 1468 [58] J. a H. van Laarhoven, M. a B. Kruff, H. Vromans, Effect of supersaturation and  
1469 crystallization phenomena on the release properties of a controlled release device based on  
1470 EVA copolymer., *J. Control. Release.* 82 (2002) 309–17.
- 1471 [59] M.R. Clark, P.F. Kiser, A. Loxley, C. McConville, R.K. Malcolm, D.R. Friend,  
1472 Pharmacokinetics of UC781-loaded intravaginal ring segments in rabbits: A comparison of  
1473 polymer matrices, *Drug Deliv. Transl. Res.* 1 (2011) 238–246.
- 1474 [60] S.R. Ugaonkar, A. Wesenberg, J. Wilk, S. Seidor, O. Mizenina, L. Kizima, et al., A novel  
1475 intravaginal ring to prevent HIV-1 , HSV-2 , HPV , and unintended pregnancy, *J. Control.*  
1476 *Release.* 213 (2015) 57–68.
- 1477 [61] J.T. Clark, M.R. Clark, N.B. Shelke, T.J. Johnson, E.M. Smith, A.K. Andreasen, et al.,

1478 Engineering a Segmented Dual-Reservoir Polyurethane Intravaginal Ring for  
 1479 Simultaneous Prevention of HIV Transmission and Unwanted Pregnancy., PLoS One. 9  
 1480 (2014) e88509.

1481 [62] P.M.M. Mesquita, R. Rastogi, T.J. Segarra, R.S. Teller, N.M. Torres, A.M. Huber, et al.,  
 1482 Intravaginal ring delivery of tenofovir disoproxil fumarate for prevention of HIV and  
 1483 herpes simplex virus infection., J. Antimicrob. Chemother. 67 (2012) 1730–8.

1484 [63] J.M. Smith, R. Rastogi, R.S. Teller, P. Srinivasan, P.M.M. Mesquita, U. Nagaraja, et al.,  
 1485 Intravaginal ring eluting tenofovir disoproxil fumarate completely protects macaques from  
 1486 multiple vaginal simian-HIV challenges., Proc. Natl. Acad. Sci. U. S. A. 110 (2013)  
 1487 16145–50.

1488 [64] S.R. Ugaonkar, J.T. Clark, L.B. English, T.J. Johnson, K.W. Buckheit, R.J. Bahde, et al.,  
 1489 An Intravaginal Ring for the Simultaneous Delivery of an HIV-1 Maturation Inhibitor and  
 1490 Reverse-Transcriptase Inhibitor for Prophylaxis of HIV Transmission, J. Pharm. Sci.  
 1491 (2015) n/a–n/a.

1492 [65] Y.A. Han, M. Singh, B.B. Saxena, Development of vaginal rings for sustained release of  
 1493 nonhormonal contraceptives and anti-HIV agents., Contraception. 76 (2007) 132–8.

1494 [66] B.B. Saxena, Y. a Han, D. Fu, P. Rathnam, M. Singh, J. Laurence, et al., Sustained release  
 1495 of microbicides by newly engineered vaginal rings., AIDS. 23 (2009) 917–22.

1496 [67] B.B. Saxena, K.E. Koldras, M. Singh, N. Nguyen, P. Rathnam, W.J. Ledger, et al.,  
 1497 Development of a Nanoporous Elastomere Intra-Vaginal Ring (IVR) for the Sustained  
 1498 Release of Non-Hormonal Contraceptives, J. Pharm. Drug Deliv. Res. 01 (2012).

1499 [68] N.T.T. Dang, H. Sivakumaran, D. Harrich, A.G. a Coombes, An Evaluation of  
 1500 Polycaprolactone Matrices for Vaginal Delivery of the Antiviral, Tenofovir, in Preventing  
 1501 Heterosexual Transmission of HIV., J. Pharm. Sci. 102 (2013) 3725–35.

1502 [69] S.M. Fetherston, L. Geer, R.S. Veazey, L. Goldman, D.J. Murphy, T.J. Ketas, et al.,  
 1503 Partial protection against multiple RT-SHIV162P3 vaginal challenge of rhesus macaques  
 1504 by a silicone elastomer vaginal ring releasing the NNRTI MC1220., J. Antimicrob.  
 1505 Chemother. 68 (2013) 394–403.

1506 [70] T. Higuchi, Mechanism of sustained-action medication. Theoretical analysis of rate of  
 1507 release of solid drugs dispersed in solid matrices, J. Pharm. Sci. 52 (1963) 1145–1149.

1508 [71] T.J. Roseman, W.I. Higuchi, Release of medroxyprogesterone acetate from a silicone  
 1509 polymer, J. Pharm. Sci. 59 (1970) 353–357.

1510 [72] I.M. Helbling, J. a Luna, M.I. Cabrera, Mathematical modeling of drug delivery from  
 1511 torus-shaped single-layer devices., J. Control. Release. 149 (2011) 258–63.

1512 [73] Y.W. Chien, H.J. Lambert, D.E. Grant, Controlled drug release from polymeric devices I:  
 1513 Technique for rapidin vitro release studies, J. Pharm. Sci. 63 (1974) 365–369.

1514 [74] Y.W. Chien, H.J. Lambert, T.K. Lin, Solution-solubility dependency of controlled release  
 1515 of drug from polymer matrix: mathematical analysis., J. Pharm. Sci. 64 (1975) 1643–7.

1516 [75] A. Nel, W. Haazen, J. Nuttall, J. Romano, Z. Rosenberg, N. van Niekerk, A safety and  
 1517 pharmacokinetic trial assessing delivery of dapivirine from a vaginal ring in healthy



1518 women, AIDS. (2014) 1.

1519 [76] A.M. Nel, W. Haazen, J.P. Nuttall, J.W. Romano, P.M.M. Mesquita, B.C. Herold, et al.,  
 1520 Pharmacokinetics and Safety Assessment of Anti-HIV Dapivirine Vaginal Microbicide  
 1521 Rings with Multiple Dosing, J. AIDS Clin. Res. 05 (2014).

1522 [77] R. Singer, P. Mawson, N. Derby, A. Rodriguez, L. Kizima, R. Menon, et al., An  
 1523 intravaginal ring that releases the NNRTI MIV-150 reduces SHIV transmission in  
 1524 macaques., Sci. Transl. Med. 4 (2012) 150ra123.

1525 [78] M. Aravantinou, R. Singer, N. Derby, G. Calenda, P. Mawson, C.J. Abraham, et al., The  
 1526 Nonnucleoside Reverse Transcription Inhibitor MIV-160 Delivered from an Intravaginal  
 1527 Ring, But Not from a Carrageenan Gel, Protects Against Simian/Human  
 1528 Immunodeficiency Virus-RT Infection, AIDS Res. Hum. Retroviruses. 28 (2012) 1467–  
 1529 1475.

1530 [79] M.R. Clark, T.J. Johnson, R.T. McCabe, J.T. Clark, A. Tuitupou, H. Elgendy, et al., A  
 1531 hot-melt extruded intravaginal ring for the sustained delivery of the antiretroviral  
 1532 microbicide UC781., J. Pharm. Sci. 101 (2012) 576–87.

1533 [80] S.M. Fetherston, P. Boyd, C.F. McCoy, M.C. McBride, K. Edwards, S. Ampofo, et al., A  
 1534 silicone elastomer vaginal ring for HIV prevention containing two microbicides with  
 1535 different mechanisms of action., Eur. J. Pharm. Sci. 48 (2012) 406–415.

1536 [81] D.J. Murphy, D. Desjardins, N. Dereuddre-Bosquet, P. Brochard, L. Perrot, A. Pruvost, et  
 1537 al., Pre-clinical development of a combination microbicide vaginal ring containing  
 1538 dapivirine and darunavir., J. Antimicrob. Chemother. (2014) 1–12.

1539 [82] Y.W. Chien, Novel drug delivery systems, 2nd Ed., Marcel Dekker, New York, 1992.

1540 [83] T.J. Johnson, M.R. Clark, T.H. Albright, J.S. Nebeker, A.L. Tuitupou, J.T. Clark, et al., A  
 1541 90-day tenofovir reservoir intravaginal ring for mucosal HIV prophylaxis., Antimicrob.  
 1542 Agents Chemother. 56 (2012) 6272–83.

1543 [84] J.M. Smith, P. Srinivasan, R.S. Teller, Y. Lo, C.T. Dinh, P.F. Kiser, et al., Tenofovir  
 1544 disoproxil fumarate intravaginal ring protects high-dose depot medroxyprogesterone  
 1545 acetate-treated macaques from multiple SHIV exposures., J. Acquir. Immune Defic. Syndr.  
 1546 68 (2015) 1–5.

1547 [85] J. Romano, B. Variano, P. Coplan, J. Van Roey, K. Douville, Z. Rosenberg, et al., Safety  
 1548 and availability of dapivirine (TMC120) delivered from an intravaginal ring., AIDS Res.  
 1549 Hum. Retroviruses. 25 (2009) 483–8.

1550 [86] M.M. Baum, I. Butkyavichene, J. Gilman, S. Kennedy, E. Kopin, A.M. Malone, et al., An  
 1551 Intravaginal Ring for the Simultaneous Delivery of Multiple Drugs, 101 (2012) 2833–  
 1552 2843.

1553 [87] J.A. Moss, A.M. Malone, T.J. Smith, I. Butkyavichene, C. Cortez, J. Gilman, et al., Safety  
 1554 and pharmacokinetics of intravaginal rings delivering tenofovir in pig-tailed macaques.,  
 1555 Antimicrob. Agents Chemother. 56 (2012) 5952–60.

1556 [88] J.A. Moss, M.M. Baum, A.M. Malone, S. Kennedy, E. Kopin, C. Nguyen, et al.,  
 1557 Tenofovir and tenofovir disoproxil fumarate pharmacokinetics from intravaginal rings.,

1558 AIDS. 26 (2012) 707–10.

1559 [89] J.A. Moss, A.M. Malone, T.J. Smith, S. Kennedy, E. Kopin, C. Nguyen, et al.,  
 1560 Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring., *Antimicrob.*  
 1561 *Agents Chemother.* 56 (2012) 875–82.

1562 [90] M.J. Keller, M. Malone, C.A. Carpenter, Y. Lo, M. Huang, L. Corey, et al., Safety and  
 1563 pharmacokinetics of acyclovir in women following release from a silicone elastomer  
 1564 vaginal ring., *J. Antimicrob. Chemother.* 67 (2012) 2005–12.

1565 [91] J.A. Moss, A.M. Malone, T.J. Smith, S. Kennedy, C. Nguyen, K.L. Vincent, et al.,  
 1566 Pharmacokinetics of a multipurpose pod-intravaginal ring simultaneously delivering five  
 1567 drugs in the ovine model., *Antimicrob. Agents Chemother.* 57 (2013) 3994–3997.

1568 [92] M. Gunawardana, M.M. Baum, T.J. Smith, J.A. Moss, An Intravaginal Ring for the  
 1569 Sustained Delivery of Antibodies., *J. Pharm. Sci.* (2014) 1–10.

1570 [93] R.J. Morrow, A.D. Woolfson, L. Donnelly, R. Curran, G. Andrews, D. Katinger, et al.,  
 1571 Sustained release of proteins from a modified vaginal ring device, *Eur. J. Pharm.*  
 1572 *Biopharm.* 77 (2011) 3–10.

1573 [94] R.S. Teller, R. Rastogi, T.J. Johnson, M.J. Blair, R.W. Hitchcock, P.F. Kiser, Intravaginal  
 1574 flux controlled pump for sustained release of macromolecules., *Pharm. Res.* 31 (2014)  
 1575 2344–53.

1576 [95] N. Promadej-Lanier, J.M. Smith, P. Srinivasan, C.F. McCoy, S. Butera, a D. Woolfson, et  
 1577 al., Development and evaluation of a vaginal ring device for sustained delivery of HIV  
 1578 microbicides to non-human primates., *J. Med. Primatol.* 38 (2009) 263–71.

1579 [96] R.K. Malcolm, R.S. Veazey, L. Geer, D. Lowry, S.M. Fetherston, D.J. Murphy, et al.,  
 1580 Sustained release of the CCR5 inhibitors CMPD167 and maraviroc from vaginal rings in  
 1581 rhesus macaques., *Antimicrob. Agents Chemother.* 56 (2012) 2251–8.

1582 [97] P. Boyd, D. Desjardins, S. Kumar, S.M. Fetherston, R. Le-Grand, N. Dereuddre-Bosquet,  
 1583 et al., A temperature-monitoring vaginal ring for measuring adherence., *PLoS One.* 10  
 1584 (2015) e0125682.

1585 [98] J.H. Lai, J.S. Hodges, Effects of processing parameters on physical properties of the  
 1586 silicone maxillofacial prosthetic materials., *Dent. Mater.* 15 (1999) 450–5.

1587 [99] I. Sivin, D.R. Mishell, F. Alvarez, V. Brache, K. Elomaa, P. Lähteenmäki, et al.,  
 1588 Contraceptive vaginal rings releasing Nestorone and ethinylestradiol: a 1-year dose-finding  
 1589 trial., *Contraception.* 71 (2005) 122–9.

1590 [100] A.D. Woolfson, R.K. Malcolm, Intravaginal drug delivery devices for the delivery of  
 1591 macromolecules and water-soluble drugs, WO/2009/003125, 2008.

1592 [101] M.M. Baum, I. Butkyavichene, S.A. Churchman, G. Lopez, C.S. Miller, T.J. Smith, et al.,  
 1593 An intravaginal ring for the sustained delivery of tenofovir disoproxil fumarate., *Int. J.*  
 1594 *Pharm.* 495 (2015) 579–87.

1595 [102] R.J.J. Groenewegen, Drug delivery system for two or more active substances, US  
 1596 5989581 A, 1999.

- 1597 [103] L.M. Ferguson, L.C. Rohan, The importance of the vaginal delivery route for  
1598 antiretrovirals in HIV prevention, *Ther. Deliv.* 2 (2011) 1535–1550.
- 1599 [104] J. W. Romano, M. Robbiani, G. F. Doncel, T. Moench, Non-Specific Microbicide Product  
1600 Development: Then and Now, *Curr. HIV Res.* 10 (2012) 9–18.
- 1601 [105] P.S. Fletcher, G.S. Wallace, P.M.M. Mesquita, R.J. Shattock, Candidate polyanion  
1602 microbicides inhibit HIV-1 infection and dissemination pathways in human cervical  
1603 explants., *Retrovirology.* 3 (2006) 46–57.
- 1604 [106] J. Balzarini, L. Van Damme, Microbicide drug candidates to prevent HIV infection,  
1605 *Lancet.* 369 (2007) 787–797.
- 1606 [107] M. Malkovsky, A. Newell, A.G. Dalgleish, Inactivation of HIV by nonoxynol-9., *Lancet*  
1607 (London, England). 1 (1988) 645.
- 1608 [108] D.R. Hicks, L.S. Martin, J.P. Getchell, J.L. Heath, D.P. Francis, J.S. McDougal, et al.,  
1609 Inactivation of HTLV-III/LAV-infected cultures of normal human lymphocytes by  
1610 nonoxynol-9 in vitro., *Lancet (London, England).* 2 (n.d.) 1422–3.
- 1611 [109] R. Jennings, A. Clegg, The inhibitory effect of spermicidal agents on replication of HSV-  
1612 2 and HIV-1 in-vitro., *J. Antimicrob. Chemother.* 32 (1993) 71–82.
- 1613 [110] R.E. Roddy, L. Zekeng, K.A. Ryan, U. Tamoufé, S.S. Weir, E.L. Wong, A controlled trial  
1614 of nonoxynol 9 film to reduce male-to-female transmission of sexually transmitted  
1615 diseases., *N. Engl. J. Med.* 339 (1998) 504–10.
- 1616 [111] M.K. Stafford, H. Ward, A. Flanagan, I.J. Rosenstein, D. Taylor-Robinson, J.R. Smith, et  
1617 al., Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects., *J.*  
1618 *Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 17 (1998) 327–31.
- 1619 [112] S.L. Hillier, T. Moench, R. Shattock, R. Black, P. Reichelderfer, F. Veronese, In vitro and  
1620 in vivo: the story of nonoxynol 9., *J. Acquir. Immune Defic. Syndr.* 39 (2005) 1–8.
- 1621 [113] Y. Haraguchi, H. Sakurai, S. Hussain, B.M. Anner, H. Hoshino, Inhibition of HIV-1  
1622 infection by zinc group metal compounds., *Antiviral Res.* 43 (1999) 123–33.
- 1623 [114] M. Arens, S. Travis, Zinc salts inactivate clinical isolates of herpes simplex virus in vitro.,  
1624 *J. Clin. Microbiol.* 38 (2000) 1758–62.
- 1625 [115] J. Kenney, A. Rodríguez, L. Kizima, S. Seidor, R. Menon, N. Jean-Pierre, et al., A  
1626 modified zinc acetate gel, a potential nonantiretroviral microbicide, is safe and effective  
1627 against simian-human immunodeficiency virus and herpes simplex virus 2 infection in  
1628 vivo., *Antimicrob. Agents Chemother.* 57 (2013) 4001–9.
- 1629 [116] T. Moulaei, K.B. Alexandre, S.R. Shenoy, J.R. Meyerson, L.R. Krumpe, B. Constantine,  
1630 et al., Griffithsin tandemers: flexible and potent lectin inhibitors of the human  
1631 immunodeficiency virus, *Retrovirology.* 12 (2015) 6.
- 1632 [117] K. Levendosky, O. Mizenina, E. Martinelli, N. Jean-Pierre, L. Kizima, A. Rodriguez, et  
1633 al., Griffithsin and Carrageenan Combination To Target Herpes Simplex Virus 2 and  
1634 Human Papillomavirus., *Antimicrob. Agents Chemother.* 59 (2015) 7290–8.
- 1635 [118] L. Zeitlin, M. Pauly, K.J. Whaley, Second-generation HIV microbicides: continued

development of griffithsin., *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 6029–30.

[119] J.A. Fernández-Romero, C. Deal, B.C. Herold, J. Schiller, D. Patton, T. Zydowsky, et al., Multipurpose prevention technologies: the future of HIV and STI protection., *Trends Microbiol.* (2015) 1–8.

[120] D. Huskens, D. Schols, Algal Lectins as Potential HIV Microbicide Candidates, *Mar. Drugs*. 10 (2012) 1476–1497.

[121] R.M. Grant, D. Hamer, T. Hope, R. Johnston, J. Lange, M.M. Lederman, et al., Whither or wither microbicides?, *Science*. 321 (2008) 532–4.

[122] S. Skoler-Karpoff, G. Ramjee, K. Ahmed, L. Altini, M.G. Plagianos, B. Friedland, et al., Efficacy of Carraguard for prevention of HIV infection in women in South Africa: a randomised, double-blind, placebo-controlled trial., *Lancet (London, England)*. 372 (2008) 1977–87.

[123] S.S.A. Karim, Q.A. Karim, Antiretroviral prophylaxis: a defining moment in HIV control., *Lancet*. 378 (2011) e23–5.

[124] P. Barditch-Crovo, S.G. Deeks, A. Collier, S. Safrin, D.F. Coakley, M. Miller, et al., Phase I/II Trial of the Pharmacokinetics, Safety, and Antiretroviral Activity of Tenofovir Disoproxil Fumarate in Human Immunodeficiency Virus-Infected Adults, *Antimicrob. Agents Chemother.* 45 (2001) 2733–2739.

[125] J. van Gelder, S. Deferme, L. Naesens, E. De Clercq, G. van den Mooter, R. Kinget, et al., Intestinal absorption enhancement of the ester prodrug tenofovir disoproxil fumarate through modulation of the biochemical barrier by defined ester mixtures., *Drug Metab. Dispos.* 30 (2002) 924–30.

[126] B.P. Kearney, J.F. Flaherty, J. Shah, Tenofovir disoproxil fumarate: clinical pharmacology and pharmacokinetics, *Clin. Pharmacokinet.* 43 (2004) 595–612.

[127] C. Lu, Y. Jia, J. Yang, Y. Song, W. Liu, Y. Ding, et al., Relative bioavailability study of a novel prodrug of tenofovir, tenofovir dipivoxil fumarate, in healthy male fasted volunteers., *Clin. Drug Investig.* 32 (2012) 333–8.

[128] U.M. Parikh, C. Dobard, S. Sharma, M. Cong, H. Jia, A. Martin, et al., Complete protection from repeated vaginal simian-human immunodeficiency virus exposures in macaques by a topical gel containing tenofovir alone or with emtricitabine., *J. Virol.* 83 (2009) 10358–65.

[129] L.C. Rohan, B.J. Moncla, R.P. Kunjara Na Ayudhya, M. Cost, Y. Huang, F. Gai, et al., In vitro and ex vivo testing of tenofovir shows it is effective as an HIV-1 microbicide., *PLoS One*. 5 (2010) e9310.

[130] G. Andrei, A. Lisco, C. Vanpouille, A. Introini, E. Balestra, J. van den Oord, et al., Topical tenofovir, a microbicide effective against HIV, inhibits herpes simplex virus-2 replication., *Cell Host Microbe*. 10 (2011) 379–89.

[131] S.S.A. Karim, A.D.M. Kashuba, L. Werner, Q.A. Karim, Drug concentrations after topical and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in women., *Lancet*. 378 (2011) 279–81.

1676 [132] J.L. Schwartz, W. Rountree, A.D.M. Kashuba, V. Brache, M.D. Creinin, A. Poindexter, et  
1677 al., A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal  
1678 candidate microbicide 1% tenofovir gel., *PLoS One*. 6 (2011) e25974.

1679 [133] M.R. Clark, D.R. Friend, Pharmacokinetics and Topical Vaginal Effects of Two  
1680 Tenofovir Gels in Rabbits, 28 (2012).

1681 [134] P.F. Kiser, A. Mahalingam, J. Fabian, E. Smith, F.R. Damian, J.J. Peters, et al., Design of  
1682 tenofovir-UC781 combination microbicide vaginal gels., *J. Pharm. Sci.* 101 (2012) 1852–  
1683 64.

1684 [135] P.A. Anton, R.D. Cranston, A. Kashuba, C.W. Hendrix, N.N. Bumpus, N. Richardson-  
1685 Harman, et al., RMP-02/MTN-006: A Phase 1 Rectal Safety, Acceptability,  
1686 Pharmacokinetic, and Pharmacodynamic Study of Tenofovir 1% Gel Compared with Oral  
1687 Tenofovir Disoproxil Fumarate, *AIDS Res. Hum. Retroviruses*. 28 (2012) 1412–1421.

1688 [136] C. Dobard, S. Sharma, A. Martin, C.-P. Pau, A. Holder, Z. Kuklenyik, et al., Durable  
1689 protection from vaginal simian-human immunodeficiency virus infection in macaques by  
1690 tenofovir gel and its relationship to drug levels in tissue., *J. Virol.* 86 (2012) 718–25.

1691 [137] V. Naranbhai, S.S.A. Karim, M. Altfeld, N. Samsunder, R. Durgiah, S. Sibeko, et al.,  
1692 Innate Immune activation enhances HIV acquisition in women, diminishing the  
1693 effectiveness of tenofovir microbicide gel., *J. Infect. Dis.* 206 (2012) 993–1001.

1694 [138] J. Nuttall, A. Kashuba, R. Wang, N. White, P. Allen, J. Roberts, et al., Pharmacokinetics  
1695 of tenofovir following intravaginal and intrarectal administration of tenofovir gel to rhesus  
1696 macaques, *Antimicrob. Agents Chemother.* 56 (2012) 103–109.

1697 [139] O. Chuchuen, M.H. Henderson, C. Sykes, M.S. Kim, A.D.M. Kashuba, D.F. Katz,  
1698 Quantitative analysis of microbicide concentrations in fluids, gels and tissues using  
1699 confocal Raman spectroscopy., *PLoS One*. 8 (2013) e85124.

1700 [140] C.W. Hendrix, B.A. Chen, V. Guddera, C. Hoesley, J. Justman, C. Nakabiito, et al.,  
1701 MTN-001: Randomized Pharmacokinetic Cross-Over Study Comparing Tenofovir Vaginal  
1702 Gel and Oral Tablets in Vaginal Tissue and Other Compartments., *PLoS One*. 8 (2013)  
1703 e55013.

1704 [141] S.S. Abdool Karim, Q. Abdool Karim, A.B.M. Kharsany, C. Baxter, A.C. Grobler, L.  
1705 Werner, et al., Tenofovir Gel for the Prevention of Herpes Simplex Virus Type 2 Infection,  
1706 *N. Engl. J. Med.* 373 (2015) 530–539.

1707 [142] F. Hladik, A. Burgener, L. Ballweber, R. Gottardo, L. Vojtech, S. Fourati, et al., Mucosal  
1708 effects of tenofovir 1% gel., *Elife*. 4 (2015) 1–12.

1709 [143] A.D.M. Kashuba, T.N. Gengiah, M.S. Epi, L. Werner, K. Yang, N.R. White, et al.,  
1710 Genital Tenofovir Concentrations Correlate With Protection Against HIV Infection in the  
1711 CAPRISA 004 Trial : Importance of Adherence for Microbicide Effectiveness, 69 (2015)  
1712 264–269.

1713 [144] A.M. Minnis, A. Van Der Straten, P. Salee, Pre-exposure Prophylaxis Adherence  
1714 Measured by Plasma Drug Level in MTN-001 : Comparison Between Vaginal Gel and  
1715 Oral Tablets in Two Geographic Regions, *AIDS Behav.* (2015).

- 1716 [145] M.R. Clark, M.M. Peet, S. Davis, G.F. Doncel, D.R. Friend, Evaluation of Rapidly  
1717 Disintegrating Vaginal Tablets of Tenofovir, Emtricitabine and Their Combination for  
1718 HIV-1 Prevention., *Pharmaceutics*. 6 (2014) 616–631.
- 1719 [146] C. McConville, D.R. Friend, M.R. Clark, K. Malcolm, Preformulation and development  
1720 of a once-daily sustained-release tenofovir vaginal tablet containing a single  
1721 excipient., *J. Pharm. Sci.* 102 (2013) 1859–68.
- 1722 [147] R. Rastogi, R.S. Teller, P.M.M. Mesquita, B.C. Herold, P.F. Kiser, Osmotic pump tablets  
1723 for delivery of antiretrovirals to the vaginal mucosa, *Antiviral Res.* (2013) 8–11.
- 1724 [148] T. Zhang, T.F. Sturgis, B.C. Youan, pH-responsive nanoparticles releasing tenofovir  
1725 intended for the prevention of HIV transmission., *Eur. J. Pharm. Biopharm.* 79 (2011)  
1726 526–36.
- 1727 [149] D. Belletti, G. Tosi, F. Forni, M. Cristina, C. Baraldi, M. Angela, et al., Chemico-physical  
1728 investigation of tenofovir loaded polymeric nanoparticles., *Int. J. Pharm.* 436 (2012) 753–  
1729 63.
- 1730 [150] T. Chaowanachan, E. Krogstad, C. Ball, K.A. Woodrow, Drug synergy of tenofovir and  
1731 nanoparticle-based antiretrovirals for HIV prophylaxis., *PLoS One*. 8 (2013) e61416.
- 1732 [151] C. Mc Conville, I. Major, D.R. Friend, M.R. Clark, A.D. Woolfson, R.K. Malcolm, et al.,  
1733 Development of polylactide and polyethylene vinyl acetate blends for the manufacture of  
1734 vaginal rings., *J. Biomed. Mater. Res. B. Appl. Biomater.* 100 (2012) 891–5.
- 1735 [152] P. Srinivasan, C. Dinh, J. Zhang, C.-P. Pau, J.M. McNicholl, Y. Lo, et al.,  
1736 Pharmacokinetic evaluation of tenofovir disoproxil fumarate released from an intravaginal  
1737 ring in pigtailed macaques after 6 months of continuous use, *J. Med. Primatol.* 43 (2014)  
1738 364–369.
- 1739 [153] B. Devlin, J. Nuttall, S. Wilder, C. Woodsong, Z. Rosenberg, Development of dapivirine  
1740 vaginal ring for HIV prevention., *Antiviral Res.* (2013).
- 1741 [154] Y. Van Herrewege, G. Vanham, J. Michiels, K. Fransen, L. Kestens, K. Andries, et al., A  
1742 Series of Diaryltriaazines and Diarylpyrimidines Are Highly Potent Nonnucleoside Reverse  
1743 Transcriptase Inhibitors with Possible Applications as Microbicides †, 48 (2004) 3684–  
1744 3689.
- 1745 [155] S. Di Fabio, J. Van Roey, G. Giannini, G. van den Mooter, M. Spada, A. Binelli, et al.,  
1746 Inhibition of vaginal transmission of HIV-1 in hu-SCID mice by the non-nucleoside  
1747 reverse transcriptase inhibitor TMC120 in a gel formulation., *AIDS*. 17 (2003) 1597–604.
- 1748 [156] J.D.S. Holt, D. Cameron, N. Dias, J. Holding, A. Muntendam, F. Oostebing, et al., The  
1749 Sheep as a Model for Preclinical Safety and Pharmacokinetic Evaluations of Candidate  
1750 Microbicides, *Antimicrob. Agents Chemother.* (2015) AAC.04954–14.
- 1751 [157] B.A. Chen, L. Panther, M.A. Marzinke, C.W. Hendrix, C.J. Hoesley, A. van der Straten,  
1752 et al., Phase 1 Safety, Pharmacokinetics, and Pharmacodynamics of Dapivirine and  
1753 Maraviroc Vaginal Rings: A Double-Blind Randomized Trial., *J. Acquir. Immune Defic.*  
1754 *Syndr.* 70 (2015) 242–9.
- 1755 [158] R.K. Malcolm, D. Lowry, P. Boyd, L. Geer, R.S. Veazey, L. Goldman, et al.,

1756 Pharmacokinetics of a CCR5 inhibitor in rhesus macaques following vaginal, rectal and  
1757 oral application., *J. Antimicrob. Chemother.* (2013) 1–5.

1758 [159] A.B. Garg, J. Nuttall, J. Romano, The future of HIV microbicides: challenges and  
1759 opportunities., *Antivir. Chem. Chemother.* 19 (2009) 143–50.

1760 [160] V. Pirrone, N. Thakkar, J.M. Jacobson, B. Wigdahl, F.C. Krebs, Combinatorial  
1761 approaches to the prevention and treatment of HIV-1 infection., *Antimicrob. Agents*  
1762 *Chemother.* 55 (2011) 1831–42.

1763 [161] J. Martinez, P. Coplan, M.A. Wainberg, Is HIV drug resistance a limiting factor in the  
1764 development of anti-HIV NNRTI and NRTI-based vaginal microbicide strategies?,  
1765 *Antiviral Res.* 71 (2006) 343–50.

1766 [162] G. Ramjee, Antiretroviral-based microbicides and oral pre-exposure prophylaxis for HIV  
1767 prevention, *Future Virol.* 6 (2011) 997–1014.

1768 [163] C. Herrera, M. Cranage, I. McGowan, P. Anton, R.J. Shattock, Reverse transcriptase  
1769 inhibitors as potential colorectal microbicides., *Antimicrob. Agents Chemother.* 53 (2009)  
1770 1797–807.

1771 [164] S.M. Schader, S.P. Colby-Germinario, J.R. Schachter, H. Xu, M.A. Wainberg, Synergy  
1772 against drug-resistant HIV-1 with the microbicide antiretrovirals, dapivirine and tenofovir,  
1773 in combination., *AIDS.* 25 (2011) 1585–94.

1774 [165] R.M. Grant, J.R. Lama, P.L. Anderson, V. McMahan, A.Y. Liu, L. Vargas, et al.,  
1775 Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men, *N.*  
1776 *Engl. J. Med.* 363 (2010) 2587–2599.

1777 [166] J.M. Marrazzo, G. Ramjee, B. a Richardson, K. Gomez, N. Mgodhi, G. Nair, et al.,  
1778 Tenofovir-Based Preexposure Prophylaxis for HIV Infection among African Women., *N.*  
1779 *Engl. J. Med.* 372 (2015) 509–518.

1780 [167] T.J. Ketas, S.M. Schader, J. Zurita, E. Teo, V. Polonis, M. Lu, et al., Entry inhibitor-based  
1781 microbicides are active in vitro against HIV-1 isolates from multiple genetic subtypes.,  
1782 *Virology.* 364 (2007) 431–40.

1783 [168] R.D. MacArthur, R.M. Novak, Reviews of anti-infective agents: maraviroc: the first of a  
1784 new class of antiretroviral agents., *Clin. Infect. Dis.* 47 (2008) 236–41.

1785 [169] J.P. Moore, D.R. Kuritzkes, A pièce de resistance: how HIV-1 escapes small molecule  
1786 CCR5 inhibitors., *Curr. Opin. HIV AIDS.* 4 (2009) 118–24.

1787 [170] C. Seibert, T.P. Sakmar, Small-molecule antagonists of CCR5 and CXCR4: a promising  
1788 new class of anti-HIV-1 drugs., *Curr. Pharm. Des.* 10 (2004) 2041–62.

1789 [171] R.S. Veazey, P.J. Klasse, S.M. Schader, Q. Hu, T.J. Ketas, M. Lu, et al., Protection of  
1790 macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell  
1791 fusion., *Nature.* 438 (2005) 99–102.

1792 [172] R.S. Veazey, T.J. Ketas, J. Dufour, T. Moroney-Rasmussen, L.C. Green, P.J. Klasse, et  
1793 al., Protection of rhesus macaques from vaginal infection by vaginally delivered  
1794 maraviroc, an inhibitor of HIV-1 entry via the CCR5 co-receptor., *J. Infect. Dis.* 202  
1795 (2010) 739–44.

1796 [173] R.K. Malcolm, C.J. Forbes, L. Geer, R.S. Veazey, L. Goldman, P. Johan Klasse, et al.,  
1797 Pharmacokinetics and efficacy of a vaginally administered maraviroc gel in rhesus  
1798 macaques., *J. Antimicrob. Chemother.* 68 (2013) 678–83.

1799 [174] A.M.J. Wensing, N.M. van Maarseveen, M. Nijhuis, Fifteen years of HIV Protease  
1800 Inhibitors: raising the barrier to resistance., *Antiviral Res.* 85 (2010) 59–74.

1801 [175] C. Herrera, R.J. Shattock, Potential use of protease inhibitors as vaginal and colorectal  
1802 microbicides., *Curr. HIV Res.* 10 (2012) 42–52.

1803 [176] J. Romano, J. Manning, A. Hemmerling, E. McGrory, B. Young Holt, Prioritizing  
1804 multipurpose prevention technology development and investments using a target product  
1805 profile., *Antiviral Res.* (2013) 1–7.

1806 [177] D. Mansour, The benefits and risks of using a levonorgestrel-releasing intrauterine system  
1807 for contraception, *Contraception.* 85 (2012) 224–234.

1808 [178] K.J. Whaley, J. Hanes, R. Shattock, R.A. Cone, D.R. Friend, Novel approaches to vaginal  
1809 delivery and safety of microbicides: biopharmaceuticals, nanoparticles, and vaccines.,  
1810 *Antiviral Res.* 88 Suppl 1 (2010) S55–66.

1811 [179] B.J. Romano, M. Brady, J. Manning, Nonclinical Development Needs and Regulatory  
1812 Requirements for Multipurpose Prevention Technologies : A Primer, (n.d.).

1813 [180] J.M. Steinbach, Protein and oligonucleotide delivery systems for vaginal microbicides  
1814 against viral STIs., *Cell. Mol. Life Sci.* 72 (2014) 469–503.

1815 [181] R.S. Veazey, R.J. Shattock, M. Pope, J.C. Kirijan, J. Jones, Q. Hu, et al., Prevention of  
1816 virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to  
1817 HIV-1 gp120., *Nat. Med.* 9 (2003) 343–6.

1818 [182] A. Sexton, S. Harman, R.J. Shattock, J.K.-C. Ma, Design, expression, and characterization  
1819 of a multivalent, combination HIV microbicide, *FASEB J.* 23 (2009) 3590–3600.

1820 [183] G. Stiegler, R. Kunert, M. Purtscher, S. Wolbank, R. Voglauer, F. Steindl, et al., A potent  
1821 cross-clade neutralizing human monoclonal antibody against a novel epitope on gp41 of  
1822 human immunodeficiency virus type 1., *AIDS Res. Hum. Retroviruses.* 17 (2001) 1757–  
1823 65.

1824 [184] A.J. Hessel, E.G. Rakasz, D.M. Tehrani, M. Huber, K.L. Weisgrau, G. Landucci, et al.,  
1825 Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human  
1826 immunodeficiency virus type 1 gp41 membrane-proximal external region protect against  
1827 mucosal challenge by simian-human immunodeficiency virus SHIVBa-L., *J. Virol.* 84  
1828 (2010) 1302–13.

1829 [185] G.C. Morris, R.C. Wiggins, S.C. Woodhall, J.M. Bland, C.R. Taylor, V. Jespers, et al.,  
1830 MABGEL 1: First Phase 1 Trial of the Anti-HIV-1 Monoclonal Antibodies 2F5, 4E10 and  
1831 2G12 as a Vaginal Microbicide, *PLoS One.* 9 (2014) e116153.

1832 [186] A. Giritch, S. Marillonnet, C. Engler, G. van Eldik, J. Botterman, V. Klimyuk, et al.,  
1833 Rapid high-yield expression of full-size IgG antibodies in plants coinfectd with  
1834 noncompeting viral vectors., *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 14701–6.

1835 [187] M.L. Radomsky, K.J. Whaley, R.A. Cone, W.M. Saltzman, Controlled vaginal delivery of



1836 antibodies in the mouse., *Biol. Reprod.* 47 (1992) 133–40.

1837 [188] J.K. Sherwood, L. Zeitlin, K.J. Whaley, R.A. Cone, M. Saltzman, Controlled release of  
 1838 antibodies for long-term topical passive immunoprotection of female mice against genital  
 1839 herpes., *Nat. Biotechnol.* 14 (1996) 468–71.

1840 [189] T.L. Wyatt, K.J. Whaley, R.A. Cone, W.M. Saltzman, Antigen-releasing polymer rings  
 1841 and microspheres stimulate mucosal immunity in the vagina., *J. Control. Release.* 50  
 1842 (1998) 93–102.

1843 [190] K.J. Whaley, J. Morton, S. Hume, E. Hiatt, B. Bratcher, V. Klimyuk, et al., Emerging  
 1844 Antibody-based Products, in: *Curr. Top. Microbiol. Immunol.*, 2012: pp. 107–126.

1845 [191] F. Villinger, Monoclonal antibody based microbicides against HIV and other STDs:  
 1846 Technological advances to a cost effective prevention, *J AIDS Clin Res.* 5 (2014) 50.

1847 [192] A. Gorlani, J. Brouwers, C. McConville, P. van der Bijl, K. Malcolm, P. Augustijns, et al.,  
 1848 Llama Antibody Fragments Have Good Potential for Application as HIV Type 1 Topical  
 1849 Microbicides, *AIDS Res. Hum. Retroviruses.* 28 (2012) 198–205.

1850 [193] J.A. Fernández-Romero, N. Teleshova, T.M. Zydowsky, M. Robbiani, Preclinical  
 1851 assessments of vaginal microbicide candidate safety and efficacy, *Adv. Drug Deliv. Rev.*  
 1852 92 (2015) 27–38.

1853 [194] L.B. Lyndgaard, R. Spångberg, C. Gilmour, C.B. Lyndgaard, F. van den Berg, A process  
 1854 analytical approach for quality control of dapivirine in HIV preventive vaginal rings by  
 1855 Raman spectroscopy, *J. Raman Spectrosc.* (2014) n/a–n/a.

1856 [195] S.E.J. Bell, A.C. Dennis, L. a Fido, R.K. Malcolm, N.M.S. Sirimuthu, C.F. Toner, et al.,  
 1857 Characterization of silicone elastomer vaginal rings containing HIV microbicide TMC120  
 1858 by Raman spectroscopy., *J. Pharm. Pharmacol.* 59 (2007) 203–7.

1859 [196] E. Gouws, B. Williams, Modelling the potential impact of an antiretroviral microbicide  
 1860 gel on the HIV epidemic in South Africa, *SACEMA Q.* (2011).

1861 [197] R. a. Ayton, G.M. Darting, A.L. Murkies, E. a. Farrell, E. Weisberg, I.S. Statistician, et  
 1862 al., A comparative study of safety and efficacy of continuous low dose oestradiol released  
 1863 from a vaginal ring compared with conjugated equine oestrogen vaginal cream in the  
 1864 treatment of postmenopausal urogenital atrophy, *BJOG An Int. J. Obstet. Gynaecol.* 103  
 1865 (1996) 351–358.

1866 [198] F.J. Roumen, T.O. Dieben, Clinical acceptability of an ethylene-vinyl-acetate  
 1867 nonmedicated vaginal ring., *Contraception.* 59 (1999) 59–62.

1868 [199] J. Vartiainen, T. Wahlström, C.G. Nilsson, Effects and acceptability of a new 17 beta-  
 1869 oestradiol-releasing vaginal ring in the treatment of postmenopausal complaints.,  
 1870 *Maturitas.* 17 (1993) 129–37.

1871 [200] F. Casper, E. Petri, Local Treatment of Urogenital Atrophy with an Estradiol-Releasing  
 1872 Vaginal Ring: A Comparative and a Placebo-Controlled Multicenter Study, *Int.*  
 1873 *Urogynecol. J. Pelvic Floor Dysfunct.* 10 (1999) 171–176.

1874 [201] E. Weisberg, I.S. Fraser, D.R. Mishell, M. Lacarra, C.W. Barding, C.W. Bardin, The  
 1875 acceptability of a combined oestrogen/progestogen contraceptive vaginal ring.,

1876 Contraception. 51 (1995) 39–44.

1877 [202] A. Faundes, E. Hardy, C. Reyes, L. Pastene, R. Portes-Carrasco, Acceptability of the  
1878 contraceptive vaginal ring by rural and urban population in two Latin American countries.,  
1879 Contraception. 24 (1981) 393–414.

1880 [203] M.R. DiMatteo, Variations in patients' adherence to medical recommendations: a  
1881 quantitative review of 50 years of research., Med. Care. 42 (2004) 200–9.

1882 [204] L. Osterberg, T. Blaschke, Adherence to medication., N. Engl. J. Med. 353 (2005) 487–  
1883 97.

1884 [205] M. Chesney, M. Chesney, Factors affecting adherence to antiretroviral therapy., Clin.  
1885 Infect. Dis. 30 Suppl 2 (2000) S171–6.

1886 [206] P.M. Ho, C.L. Bryson, J.S. Rumsfeld, Medication adherence: Its importance in  
1887 cardiovascular outcomes, Circulation. 119 (2009) 3028–3035.

1888 [207] E.E. Tolley, P.F. Harrison, E. Goetghebeur, K. Morrow, R. Pool, D. Taylor, et al.,  
1889 Adherence and its measurement in phase 2/3 microbicide trials., AIDS Behav. 14 (2010)  
1890 1124–36.

1891 [208] R.A. Ferrer, K.M. Morrow, W.A. Fisher, J.D. Fisher, Toward an information–motivation–  
1892 behavioral skills model of microbicide adherence in clinical trials, AIDS Care. 22 (2010)  
1893 997–1005.

1894 [209] K.R. Amico, L.E. Mansoor, A. Corneli, K. Torjesen, A. Van Der Straten, Adherence  
1895 support approaches in biomedical HIV prevention trials: Experiences, insights and future  
1896 directions from four multisite prevention trials, AIDS Behav. 17 (2013) 2143–2155.

1897 [210] L.E. Mansoor, Q. Abdool Karim, N. Yende-Zuma, K.M. MacQueen, C. Baxter, B.T.  
1898 Madlala, et al., Adherence in the CAPRISA 004 tenofovir gel microbicide trial., AIDS  
1899 Behav. 18 (2014) 811–9.

1900 [211] K.M. MacQueen, M.A. Weaver, F. van Loggerenberg, S. Succop, N. Majola, D. Taylor, et  
1901 al., Assessing adherence in the CAPRISA 004 tenofovir gel HIV prevention trial: results  
1902 of a nested case-control study., AIDS Behav. 18 (2014) 826–32.

1903 [212] K.T. Mngadi, S. Maarschalk, A.C. Grobler, L.E. Mansoor, J.A. Frohlich, B. Madlala, et  
1904 al., Disclosure of microbicide gel use to sexual partners: influence on adherence in the  
1905 CAPRISA 004 trial., AIDS Behav. 18 (2014) 849–54.

1906 [213] L. Van Damme, A. Corneli, K. Ahmed, K. Agot, J. Lombaard, S. Kapiga, et al.,  
1907 Preexposure Prophylaxis for HIV Infection among African Women, N. Engl. J. Med. 367  
1908 (2012) 411–422.

1909 [214] C. Celum, J.M. Baeten, Tenofovir-based pre-exposure prophylaxis for HIV prevention,  
1910 Curr. Opin. Infect. Dis. 25 (2012) 51–57.

1911 [215] S.M. McCormack, M. Gafos, M. Desai, M.S. Cohen, Biomedical prevention: state of the  
1912 science., Clin. Infect. Dis. 59 Suppl 1 (2014) S41–6.

1913 [216] K.S. Ingersoll, J. Cohen, The impact of medication regimen factors on adherence to  
1914 chronic treatment: a review of literature., J. Behav. Med. 31 (2008) 213–24.

- 1915 [217] M.E. Kruk, N. Schwalbe, The relation between intermittent dosing and adherence:  
1916 preliminary insights., *Clin. Ther.* 28 (2006) 1989–95.
- 1917 [218] A. Graziottin, Safety, efficacy and patient acceptability of the combined estrogen and  
1918 progestin transdermal contraceptive patch: a review., *Patient Prefer. Adherence.* 2 (2008)  
1919 357–67.
- 1920 [219] J. Trussell, Contraceptive failure in the United States., *Contraception.* 83 (2011) 397–404.
- 1921 [220] A.R.C. Trial, M.L. Gilliam, A. Neustadt, M. Kozloski, S. Mistretta, S. Tilmon, et al.,  
1922 Adherence and acceptability of the contraceptive ring compared with the pill among  
1923 students: a randomized controlled trial., *Obstet. Gynecol.* 115 (2010) 503–510.
- 1924 [221] E.T. Montgomery, A. van der Straten, H. Cheng, L. Wegner, G. Masenga, C. von  
1925 Mollendorf, et al., Vaginal ring adherence in sub-Saharan Africa: expulsion, removal, and  
1926 perfect use., *AIDS Behav.* 16 (2012) 1787–98.
- 1927 [222] E. Hardy, E.M. Hebling, M.H. Sousa, A.F. Almeida, E. Amaral, Delivery of microbicides  
1928 to the vagina: difficulties reported with the use of three devices, adherence to use and  
1929 preferences., *Contraception.* 76 (2007) 126–31.
- 1930 [223] K.M. Macqueen, E.E. Tolley, D.H. Owen, K.R. Amico, K.M. Morrow, T. Moench, et al.,  
1931 Debate article An interdisciplinary framework for measuring and supporting adherence in  
1932 HIV prevention trials of ARV-based vaginal rings, 17 (2014) 1–7.
- 1933 [224] C. Woodsong, K. MacQueen, K.R. Amico, B. Friedland, M. Gafos, L. Mansoor, et al.,  
1934 Microbicide clinical trial adherence: insights for introduction., *J. Int. AIDS Soc.* 16 (2013)  
1935 18505.
- 1936 [225] M.J. Stirratt, J. Dunbar-Jacob, H.M. Crane, J.M. Simoni, S. Czajkowski, M.E. Hilliard, et  
1937 al., Self-report measures of medication adherence behavior: recommendations on optimal  
1938 use, *Transl. Behav. Med.* 5 (2015) 470–482.
- 1939 [226] C.K. Mauck, A. van der Straten, Using objective markers to assess participant behavior in  
1940 HIV prevention trials of vaginal microbicides., *J. Acquir. Immune Defic. Syndr.* 49 (2008)  
1941 64–9.
- 1942 [227] J. Shen, D.J. Burgess, Accelerated in-vitro release testing methods for extended-release  
1943 parenteral dosage forms., *J. Pharm. Pharmacol.* 64 (2012) 986–96.
- 1944 [228] J.A. Moss, P. Srinivasan, T.J. Smith, I. Butkyavichene, G. Lopez, A.A. Brooks, et al.,  
1945 Pharmacokinetics and Preliminary Safety Study of Pod-Intravaginal Rings Delivering  
1946 Antiretroviral Combinations for HIV Prophylaxis in a Macaque Model, *Antimicrob.*  
1947 *Agents Chemother.* 58 (2014) 5125–5135.
- 1948 [229] C. McConville, J.M. Smith, C.F. McCoy, P. Srinivasan, J. Mitchell, A. Holder, et al.,  
1949 Lack of in vitro-in vivo correlation for a UC781-releasing vaginal ring in macaques., *Drug*  
1950 *Deliv. Transl. Res.* 5 (2015) 27–37.
- 1951 [230] D.F. Katz, A. Yuan, Y. Gao, Vaginal drug distribution modeling., *Adv. Drug Deliv. Rev.*  
1952 92 (2015) 2–13.
- 1953 [231] R.M. Machado, A. Palmeira-de-Oliveira, C. Gaspar, J. Martinez-de-Oliveira, R. Palmeira-  
1954 de-Oliveira, Studies and methodologies on vaginal drug permeation., *Adv. Drug Deliv.*

1955 Rev. 92 (2015) 14–26.

1956 [232] T.J. Johnson, P. Srinivasan, T.H. Albright, K. Watson-Buckheit, L. Rabe, A. Martin, et  
1957 al., Safe and sustained vaginal delivery of pyrimidinedione HIV-1 inhibitors from  
1958 polyurethane intravaginal rings., *Antimicrob. Agents Chemother.* 56 (2012) 1291–9.

1959 [233] M. Hsu, B.F. Keele, M. Aravantinou, N. Krawczyk, S. Seidor, C.J. Abraham, et al.,  
1960 Exposure to MIV-150 from a High-Dose Intravaginal Ring Results in Limited Emergence  
1961 of Drug Resistance Mutations in SHIV-RT Infected Rhesus Macaques., *PLoS One.* 9  
1962 (2014) e89300.

1963 [234] L.A. Ouattara, P. Barnable, P. Mawson, S. Seidor, T.M. Zydowsky, L. Kizima, et al.,  
1964 MIV-150-containing intravaginal rings protect macaque vaginal explants against SHIV-RT  
1965 infection., *Antimicrob. Agents Chemother.* 58 (2014) 2841–8.

1966

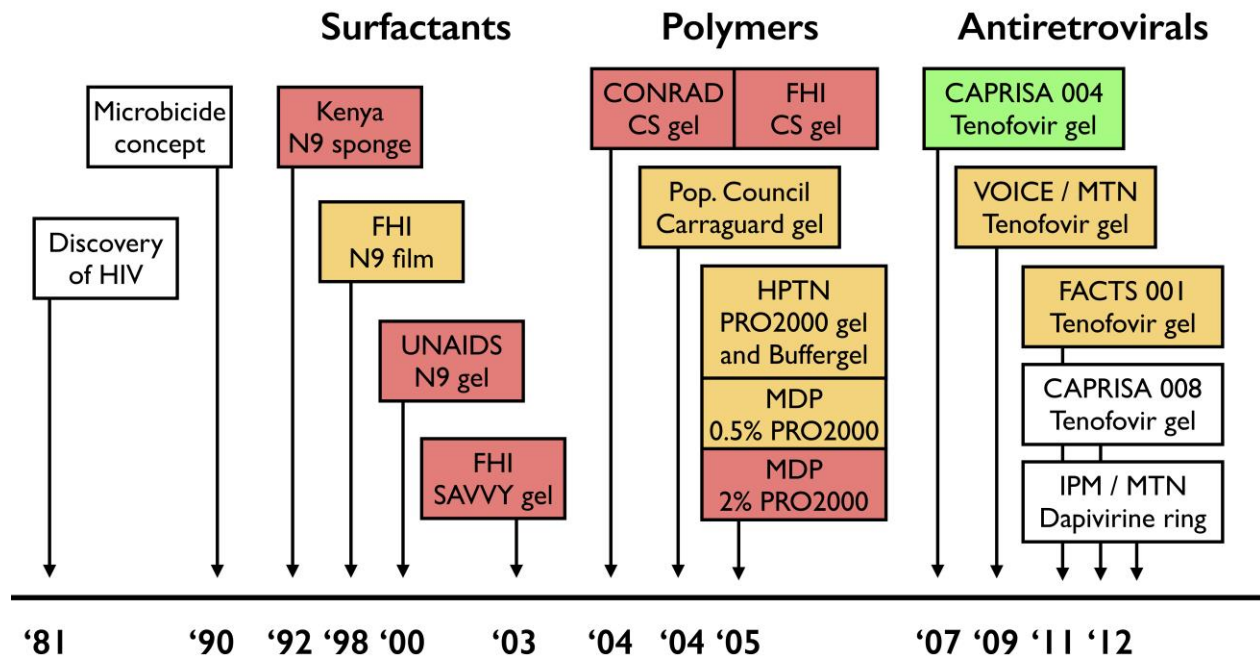
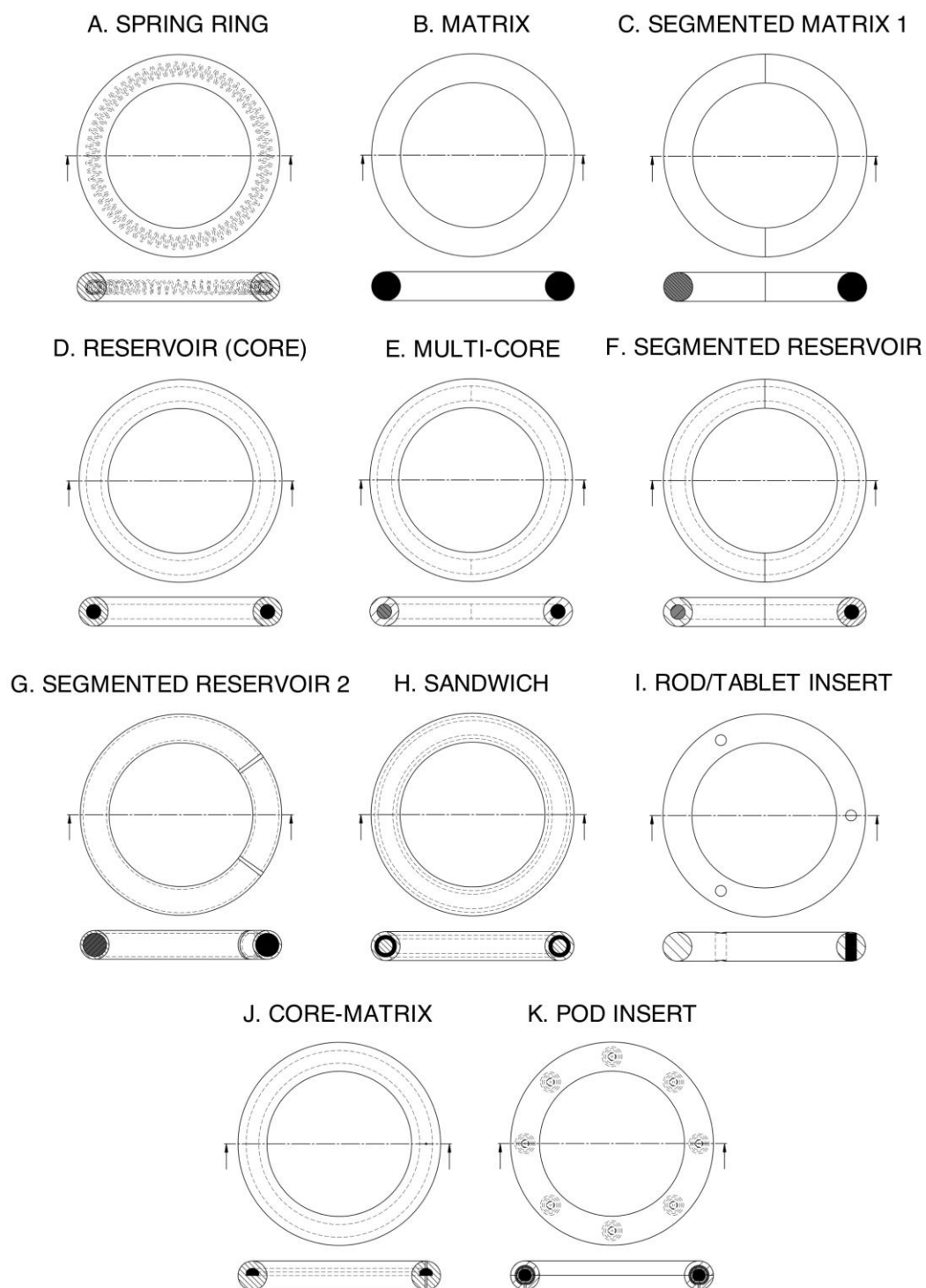


Figure 1. Summary timeline describing key moments, and particularly major clinical activities (study start dates), in the development of HIV microbicides. Red boxes represent clinical studies that reported an increase in HIV acquisition with use of the microbicide test product. Orange boxes represent trials in which the microbicide test product showed no protective effect. Green box represents a microbicide test product that offered moderate protection. White boxes at the right of the figure represent studies that are ongoing. CS – cellulose sulfate; FHI – Family Health International (now FHI 360); IPM – International Partnership for Microbicides; MDP – Microbicides Development Programme; MTN – Microbicides Trial Network; N9 – nonoxynol-9.



1977

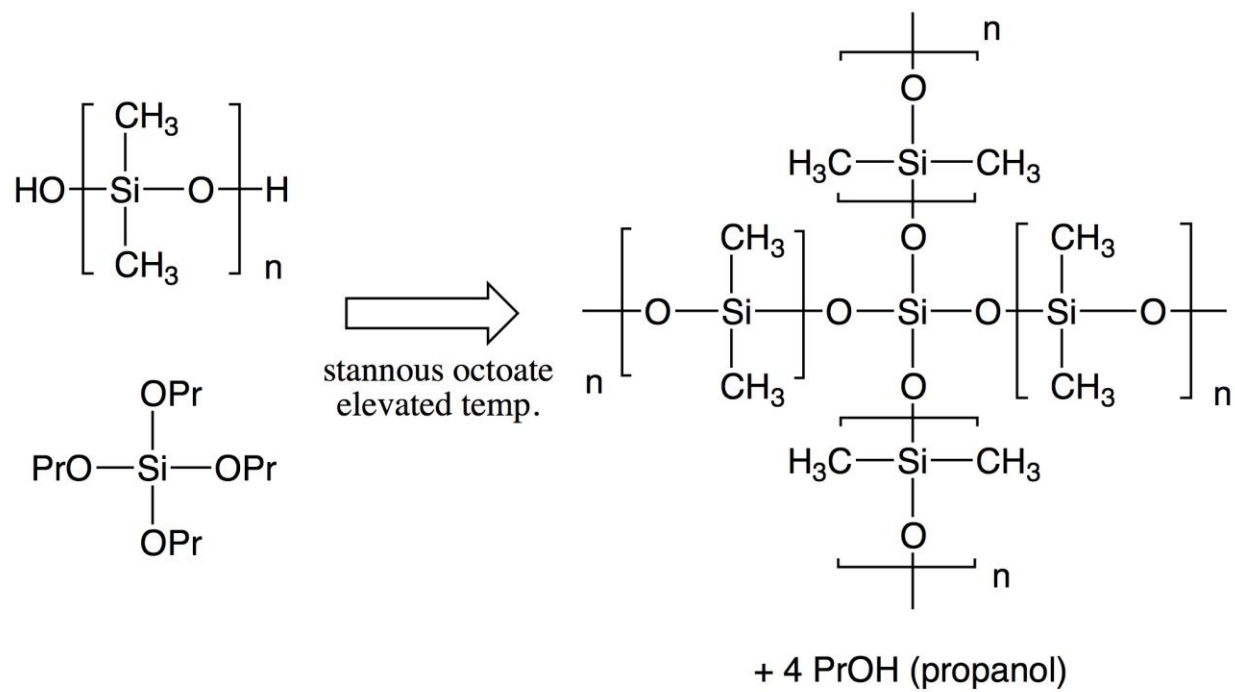
1978

1979

1980

1981

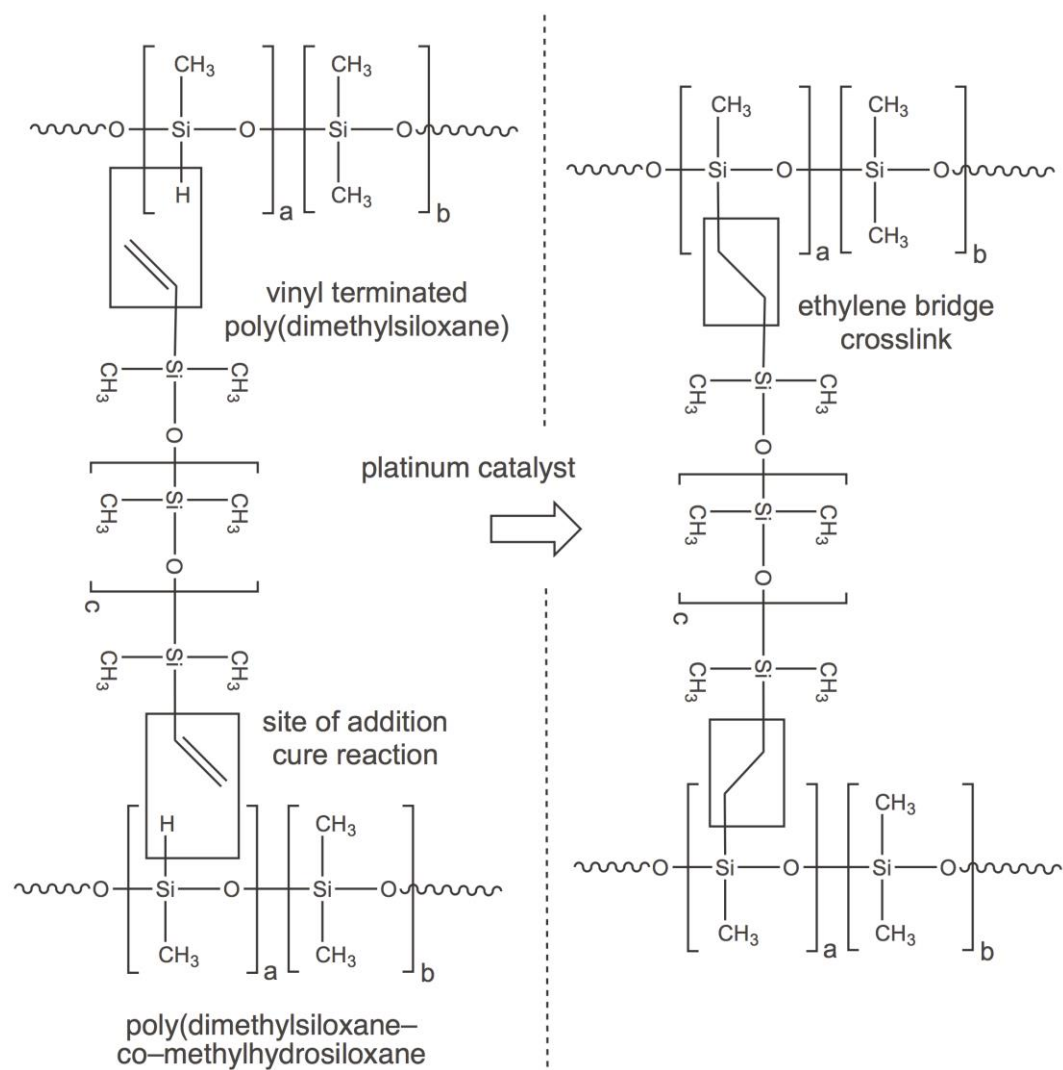
Figure 2. Full ring (upper) and cross-sectional (lower) views of the various vaginal ring designs reported in the scientific literature for the delivery of HIV microbicides. Dark shading represents the location of the active agent(s).



1982

1983 Figure 3. Curing reaction for condensation-cure silicone elastomer systems.

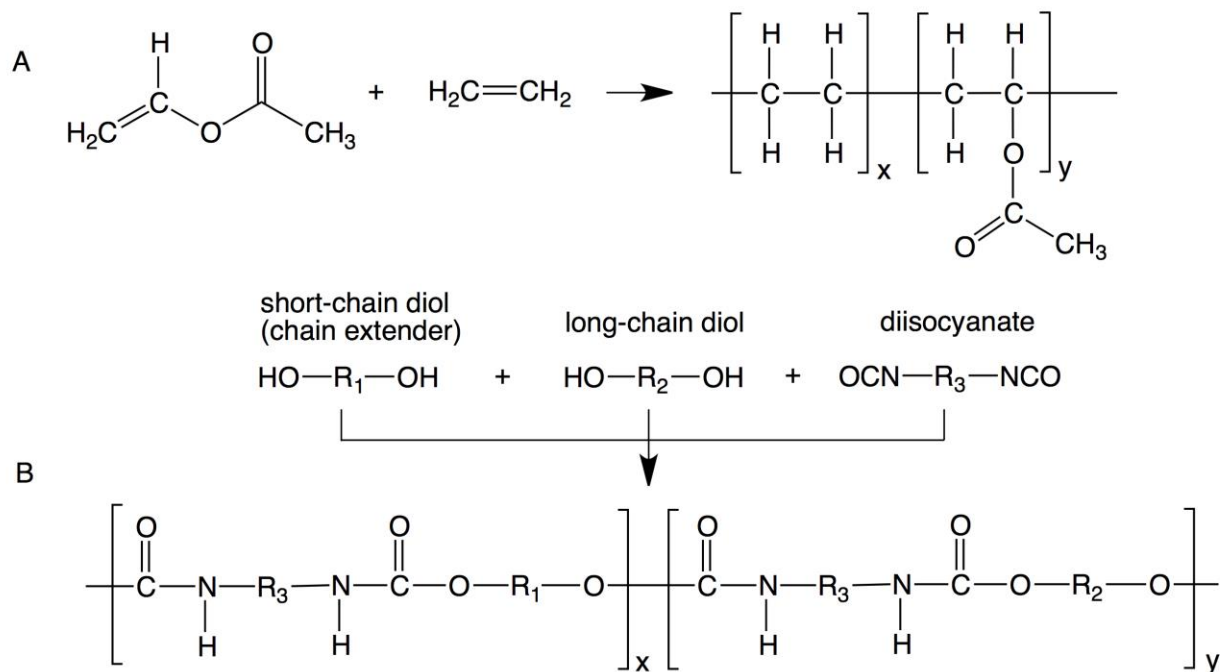
1984



1985

1986 Figure 4. Representation of the platinum-catalysed hydrosilylation reaction for cure of addition-  
 1987 cure silicone elastomer systems.





1988  
 1989 Figure 5. General synthetic reactions and representative chemical structures for (A)  
 1990 poly(ethylene)-co-vinyl acetate (EVA) polymers and (B) polyurethanes used in the fabrication of  
 1991 thermoplastic vaginal rings.

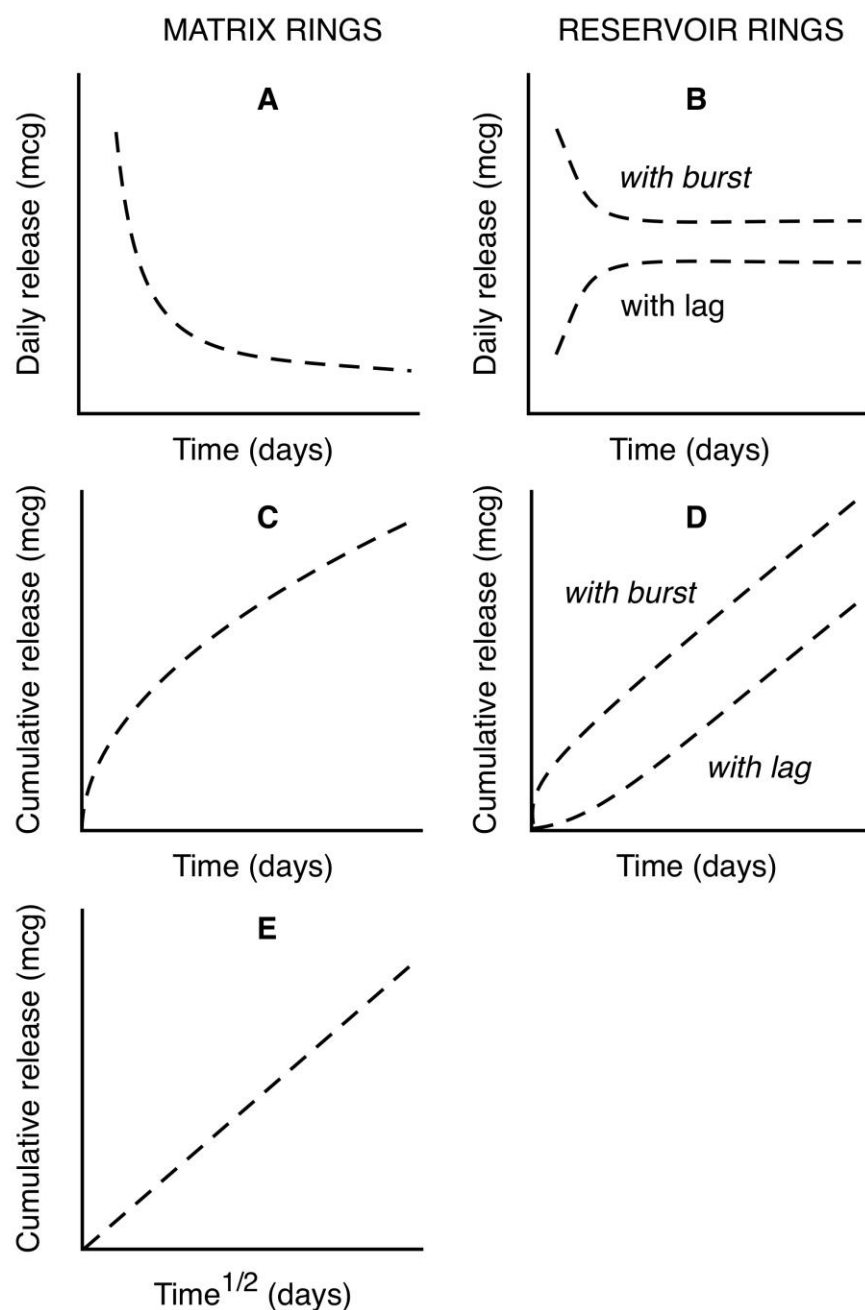


Figure 6. Representative daily and cumulative drug release vs. time profiles for non-degradable, non-swelling matrix-type and reservoir-type vaginal rings. Matrix-type rings contain crystalline drug distributed throughout the entire ring body and exhibit root time kinetics. Here, reservoir rings can refer to either a conventional reservoir-type ring comprising one or more drug cores encapsulated by a non-medicated membrane (Figure 2 D–G), a core-matrix ring (Figure 2 J) or a pod insert type ring (Figure 2 K), all of which display (pseudo) zero-order drug release kinetics.

## Silicone Elastomer Ring Manufacture

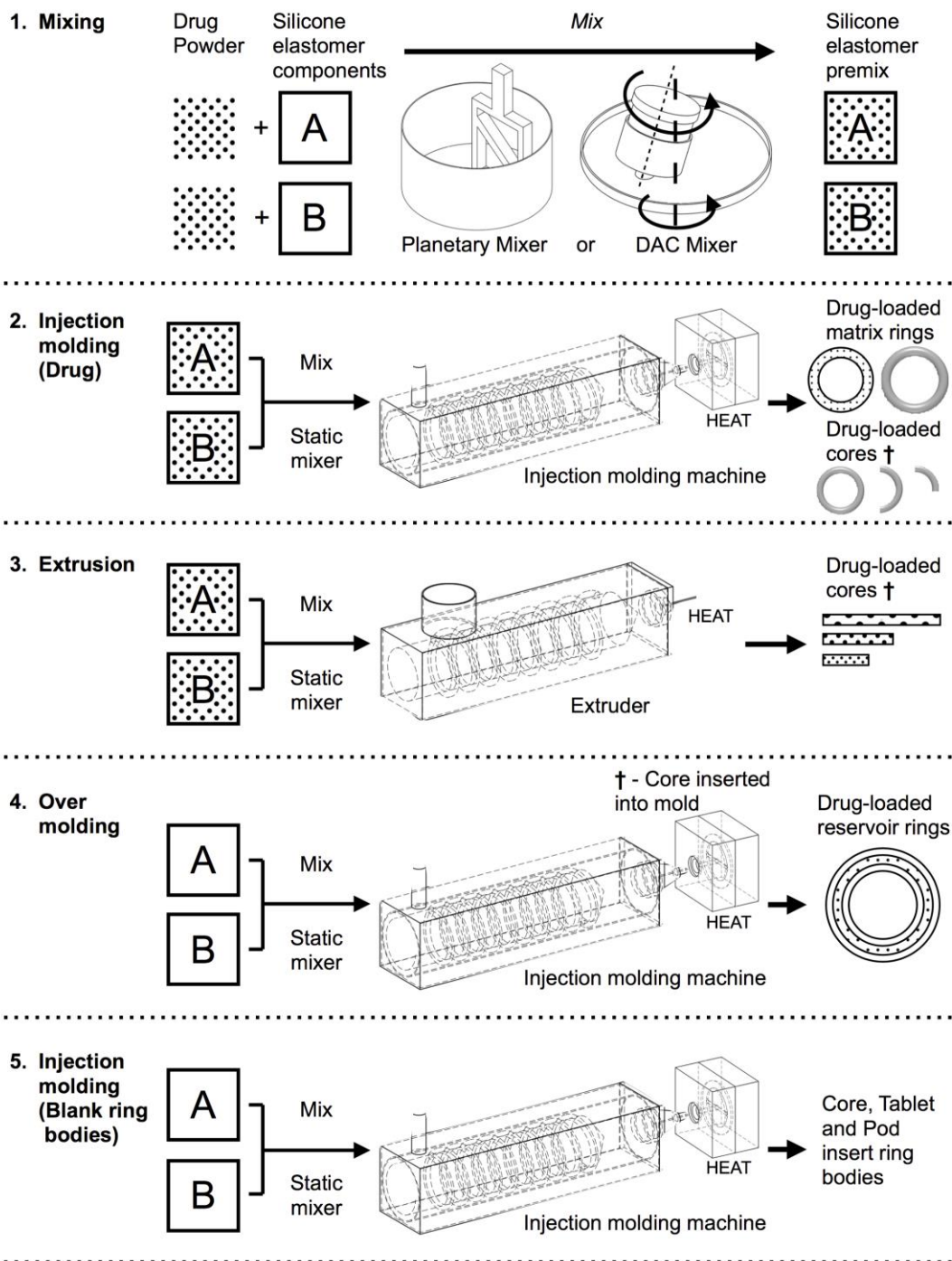


Figure 7. Representative steps in the manufacturing process for fabrication of silicone elastomer vaginal rings.

## Thermoplastic Ring Manufacture

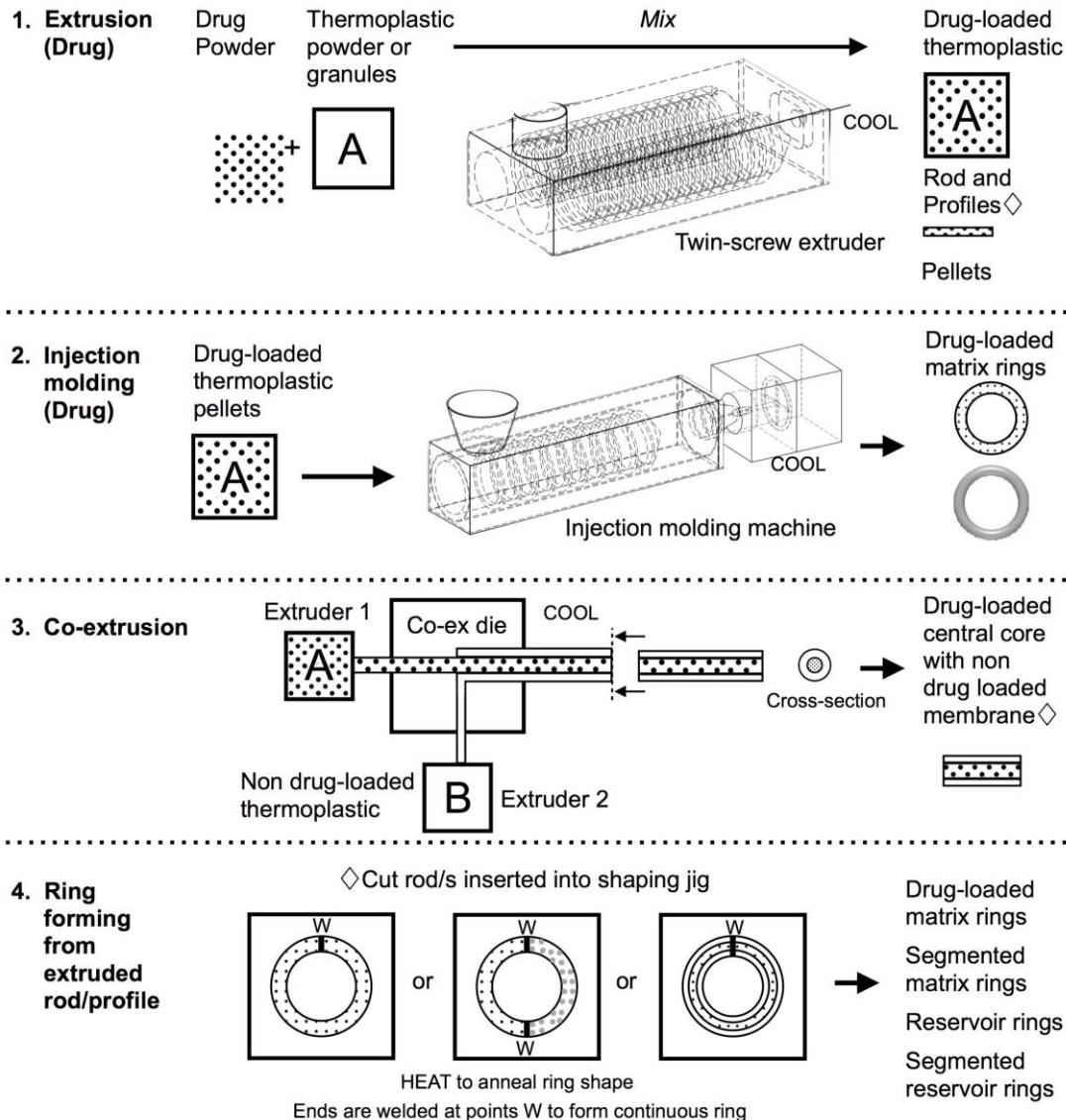
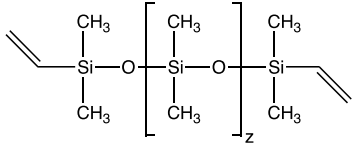
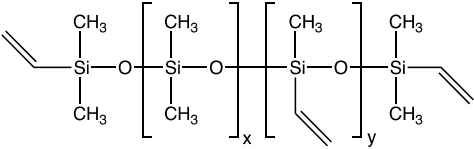
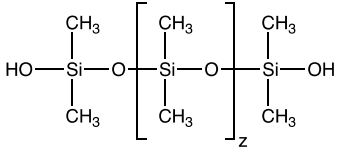
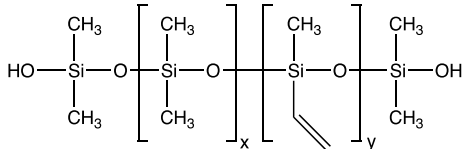


Figure 8. Representative steps in the manufacturing process for fabrication of thermoplastic vaginal rings.

2007 Table 1. Representative composition of the base material for an addition-cure silicone elastomer  
 2008 system. PDMS – polydimethylsiloxane.

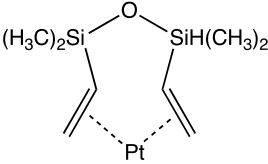
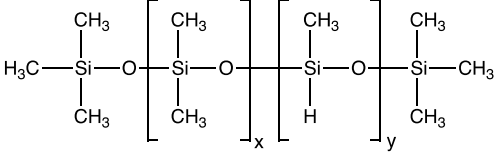
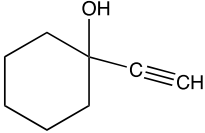
2009

Silicone base component	Chemical structure	Typical conc.
terminal dimethylvinyl PDMS		35%
terminal dimethylvinyl + internal vinyl PDMS		35% y=0.2%
hydroxy-terminated PDMS oil		5%
hydroxy-terminated + internal vinyl PDMS oil		5%
reinforcing fused silica	SiO <sub>2</sub>	20%

2010

2011 Table 2. Representative Part A and B formulation components of addition-cure silicone elastomer  
2012 system used in the fabrication of vaginal rings.

2013

Component	Representative chemical structure	Part
silicone elastomer base	(see Table 1)	A and B
platinum-based hydrosilylation catalyst		A
hydride crosslinker		B
inhibitor (used to control work time)		B

2014

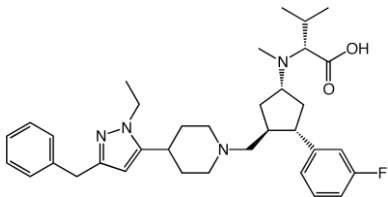
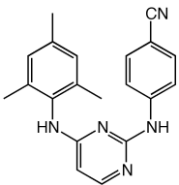
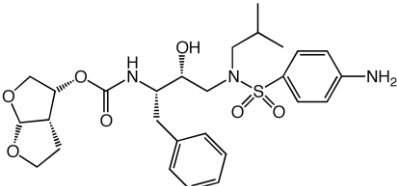
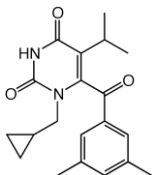
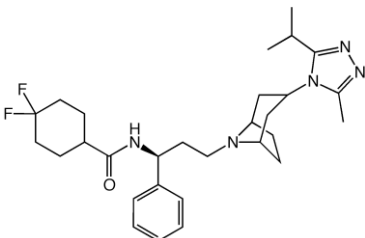
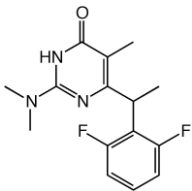
2015

2016 Table 3. Summary of non-ARV HIV microbicide candidates that have been formulated in vaginal ring devices.

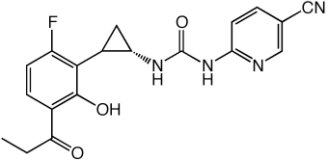
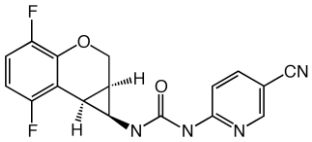
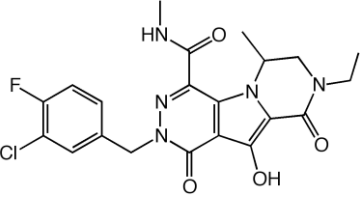
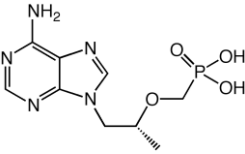
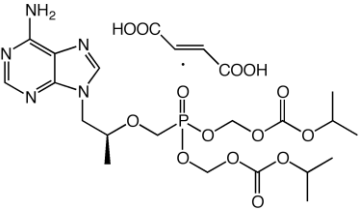
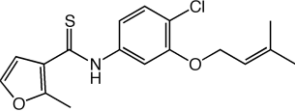
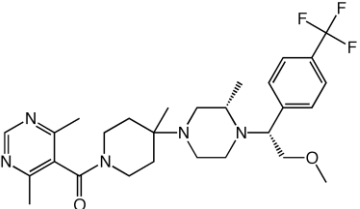
2017

Microbicide Candidates / APIs	Ring type / polymer	Clinical indications				Organization	Development stage
		HIV	HSV-2	HPV	Pregnancy		
Nonoxynol-9 (N-9)	Matrix / silicone elastomer	✓				Queen's University Belfast	Halted
Zinc acetate, carrageenan (ZC)	Core / EVA	✓	✓	✓		Population Council	Advanced Preclinical
Zinc acetate, carrageenan, MIV-150 (MZC)	Core / EVA	✓	✓	✓		Population Council	Advanced Preclinical
Zinc acetate, MIV-150, LNG (MZL)	Core / EVA	✓			✓	Population Council & ProMed Pharma	Early Preclinical
Zinc acetate, carrageenan, MIV-150, LNG (MZCL)	Core / EVA	✓	✓	✓	✓		Early Preclinical
Boc-lysinated betulonic acid (Boc-LBA)	Reservoir / Bio-soluble acacia gum	✓				Weill-Cornell Medical College & BioRing LLC	n/a
Biorings™; Boc-lysinated betulonic acid, ferrous gluconate, ascorbic acid, polyamino-polycarboxylic acid, TFV	Nanoporous elastomer (hydrophilic) hydrogel	✓			✓	Biorings LLC	Early Preclinical
Griffithsin	n/a	✓	✓	✓		Population Council	Early Preclinical

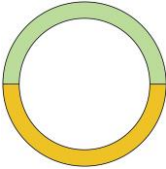
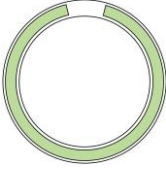
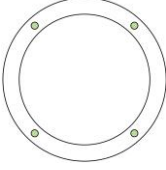
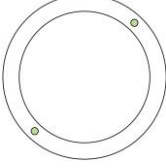
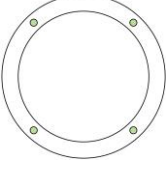
2018 Table 4. Summary of antiretroviral drugs that have been formulated in vaginal rings as HIV  
 2019 microbicides. ENT – entry inhibitor; INT – integrase inhibitor; NNRTI – non-nucleoside reverse  
 2020 transcriptase inhibitor; PI – protease inhibitor; NRTI – nucleoside reverse transcriptase inhibitor.  
 2021

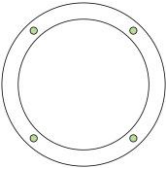
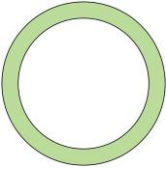
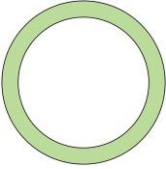
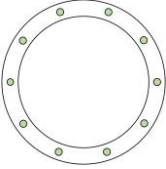
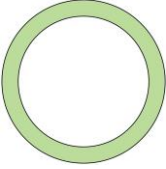
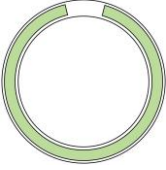
Antiretroviral	Mechanism of action	Chemical structure	Vaginal ring types	Reference(s)
CMPD167	ENT		Matrix; silicone elastomer	[96,158]
dapivirine	NNRTI		Matrix and core; silicone elastomer	[20,38,56,75,76,80,81,153,195]
DRV	PI		Matrix; silicone elastomer	[81]
IQP-0528	NNRTI		Matrix; polyurethane	[64,232]
maraviroc	ENT		Matrix; silicone elastomer	[80,96]
MC1220	NNRTI		Matrix; silicone elastomer	[69]



MIV-150	NNRTI		Matrix; silicone elastomer and EVA	[77,233,234]
MIV-160	NNRTI		Matrix (solvent cast)	[78]
MK-2048	INT		reservoir; also being evaluated in combination with vicriviroc	n/a
tenofovir	NRTI		pod; reservoir	[61,83,86,87,89, 91]
tenofovir disoproxil fumarate	NRTI		matrix, reservoir and pod; TPU, EVA and silicone elastomer	[62,63,84,88,15 2]
UC781	NNRTI		matrix; EVA, TPU and silicone elastomer	[41,79,229]
vicriviroc	ENT		reservoir; also being evaluated in combination with MK-2048	n/a

2023 Table 5. Summary of vaginal rings reported for the delivery of tenofovir (TFV) or tenofovir disoproxil fumarate (TDF). Abbreviations:  
 2024 ACV – acyclovir; E2 – estradiol; ETN – etonogestrel; LNG – levonorgestrel; NVP – nevirapine; PK – pharmacokinetic; SQV –  
 2025 saquinavir; TPU – polyurethane.  
 2026

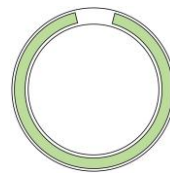
Drug(s)	Vaginal ring type	Materials	Image	Study details	Reference
TFV + DPV	segmented matrix	hydrophilic and hydrophobic TPUs		<i>in vitro</i> characterization of dual segment polyurethane VRs; 30 day release of TFV and DPV achieved	[43]
TFV	reservoir (tubing) filled with either solid TFV or TFV+glycerol+ water paste	hydrophilic TPUs		<i>in vitro</i> characterization; PK testing in sheep; 90 day of TFV achieved	[83]
TFV + ACV	pod (multiple)	silicone elastomer ring body; polylactic acid-coated pellets		<i>in vitro</i> characterization; 28 day of both TFV and ACV achieved	[86]
TFV and TDF	pod (x2)	silicone elastomer ring body; polylactic acid-coated pellets		28-day PK study in sheep; tissue levels of TDF were 86-fold higher than TFV	[88]
TFV + ACV	pod (x4)	silicone elastomer ring body; polylactic acid-coated pellets		<i>in vitro</i> characterization; 28-day PK evaluation in rabbits and sheep	[89]

TFV	pod (×4)	silicone elastomer ring body; polylactic acid-coated pellets		<i>in vitro</i> characterization; safety and 28-day PK evaluation in pig-tailed macaques	[87]
TDF	matrix	hydrophilic TPU; EVA; silicone elastomer		<i>in vitro</i> characterization, including testing in cell and explant models	[62]
TFV	matrix	PLA and EVA blends		<i>in vitro</i> characterization	[151]
TFV + NVP + SQV + E2 + ETG	pod (×10)	silicone elastomer ring body; polylactic acid-coated pellets		28-day PK study in sheep; demonstration that five different drugs can be administered simultaneously	[91]
TFV	matrix	PCL		<i>in vitro</i> characterization	[68]
TDF	matrix; reservoir (tubing) with solid TDF ± solid excipients	hydrophilic TPU		<i>in vitro</i> characterization; multiple low-dose SHIV challenge study in macaques; 100% protection achieved	[63]

TDF

reservoir

NA



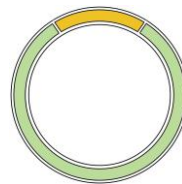
6-month safety and PK  
study in pig-tailed  
macaques

[152]

TFV + LNG

dual-segment reservoir

hydrophilic TPU



*in silico*, *in vitro* and *in vivo* (rabbit) evaluation

[61]

2028 Table 6. Completed, ongoing and planned microbicide vaginal ring clinical trials. Abbreviations  
 2029 used in table: DPV – dapivirine; IPM – International Partnership for Microbicides; FTC –  
 2030 emtricitabine; PK – pharmacokinetics; LNG – levonorgestrel; MTN – Microbicide Trials  
 2031 Network; MPT – multipurpose prevention technology; MVC – maraviroc; TDF – Tenofovir  
 2032 disoproxil fumarate; TFV – tenofovir.  
 2033

Trial	Description	Phase	Countries	No. women	Status
IPM 001	DPV ring safety (Ring-001)	1	Belgium	12	Completed
IPM 008	DPV ring safety (Ring-002)	1	Belgium	13	Completed
IPM 011	Placebo ring safety & acceptability	n/a	South Africa / Tanzania	170	Completed
IPM 013	DPV ring PK (Ring-004)	1	Belgium	48	Completed
IPM 015	DPV ring safety (Ring-004)	1/2	Kenya, Malawi, South Africa, Tanzania	280	Completed
IPM 018	DPV ring PK (Ring-002 & Ring-003)	1	Belgium	24	Completed
IPM 024	DPV ring PK (Ring-004)	1	Belgium	16	Completed
IPM 026 / MTN 013	MVC, DPV, and DPV-MVC combination rings	1	USA	48	Completed
IPM 027	'The Ring Study' – DPV ring long-term safety and efficacy	3	South Africa, Uganda	1959	Ongoing
IPM 028	DPV ring drug-drug interaction (Ring-004)	1	Belgium	36	Completed
IPM 029	DPV ring & male condom functionality (Ring-004)	n/a	USA	70 couples	Completed
IPM 030 / MTN 023	DPV ring safety (Ring-004)	2a	USA	96	Ongoing
IPM 031 / MTN 024	DPV ring safety and acceptability (Ring-004)	2a	USA	96	Ongoing
IPM 033	DPV ring and female condom functionality (Ring-004)	n/a	USA	80 couples	Study report in progress
IPM 034	DPV ring PK (Ring-004)	n/a	Belgium	40	Completed
IPM 035	DPV ring & menses and tampon use (Ring-004)	n/a	Belgium	32	Ongoing
IPM 036	DPV ring drug-drug interaction (Ring-004)	1	Belgium	36	Ongoing

MTN 020	ASPIRE – DPV ring efficacy & safety (Ring-004)	3	Malawi, South Africa, Uganda, Zambia, Zimbabwe	3475	Completed; awaiting results
IPM	Combination MPT ring containing DPV+LNG	1	n/a	n/a	Planned (2016)
CONRAD	TDF ring / safety and PK (IVR-001)	1	USA	30	Completed
CONRAD	TFV-only ring and TFV+LNG ring / Safety, PK & PD	1	USA, Dominican Republic	100	Ongoing
Auritec	TDF-only, TDF+FTC and TDF+FTC+MRV rings / Safety and PK	0	USA	6	Ongoing
MTN 027 / NIAID	MPT rings containing Vicriviroc and MK-2048A / Safety and PK	1	USA	48	Recruiting
MTN 028 / NIAID	MPT rings containing Vicriviroc and MK-2048A / PK	1	USA	18	Recruiting
Population Council	MPT ring containing griffithsin	1	n/a	n/a	Planned (2017/18)

2035 Table 7. List of published articles describing animal testing of microbicide vaginal rings.

2036

Animal species	Compound(s) tested	Reference
Macaque (cynomolgus)	DPV + DRV	[81]
Macaque (pigtail)	IQP-0528; IQP-0532	[232]
	TDF	[63,84,152]
	TDF + FTC + MVC	[228]
	UC781	[229]
Macaque (rhesus)	CMPD167	[96,158]
	MC1220	[69]
	MIV-150	[77,233,234]
	MIV-150 + ZA + CG + LNG	[60]
	MIV-160	[78]
	MRV	[96]
Rabbits	TFV + ACV	[89]
	TFV + LNG	[61]
	UC781	[79]
Sheep	DPV	[156]
	TFV; TDF	[83,88]
	TFV + ACV	[89]
	TFV + NVR + SQN + ETN + EST	[91]

2037

Table 8. Values for the vaginal fluid and plasma/serum concentration of DPV at various time points after initial ring insertion, compared with daily release values measured *in vitro* (into IPA:H<sub>2</sub>O) at equivalent times.

Compartment	Species	Time (days)			
		4	8	15	22
Plasma/serum (pg/mL)	Human <sup>a</sup>	299	357	357	327
	Sheep <sup>b</sup>	58	59	37	32
	Macaque <sup>c,d</sup>	164	94	78	110
Vaginal fluid (µg/g)	Human <sup>a</sup>	44	45	44	37
	Sheep <sup>b</sup>	4.2	2.6	1.7	1
	Macaque <sup>c,d</sup>	3.7	6.1	6	4.9
<i>In vitro</i> daily release (µg/day) <sup>e</sup>	Human ring	684	425	273	212
	Macaque ring	416	278	201	156

<sup>a</sup> Values estimated from published graphs of plasma and vaginal fluid concentration against time; weighted by the number of participants [75,76]

<sup>b</sup> Values estimated from published graphs of plasma and vaginal fluid concentration against time [156]

<sup>c</sup> Values interpolated from concentrations measured at time points either side of the time in question [81]

<sup>d</sup> Macaque sized rings (25×6 mm) utilising an alternative platinum catalysed silicone were used.

<sup>e</sup> *In vitro* release was measured into IPA:H<sub>2</sub>O, 100 mL for human sized rings, 50 mL for macaque rings.